



Faculty of Agriculture
University of Banja Luka



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AGRICULTURAL SCIENCES**



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EDITORIAL

Global agricultural production is currently navigating a complex landscape of interconnected challenges, spanning from the accelerating impacts of climate change to technological, social, and economic barriers. Overcoming these obstacles is paramount to ensuring food security for a growing global population, and the ultimate key to achieving this resilience lies in knowledge. Scientific research serves as the cornerstone for generating new insights, offering innovative pathways to adapt food and feed production systems to rapidly changing environmental and market conditions.

In the context of Southeast Europe, strategic frameworks consistently emphasize that a knowledge-based economy must serve as the foundation for agricultural advancement, necessitating sustained investment in research and innovation. Within the Republic of Srpska, Bosnia and Herzegovina, and the broader region, these innovations must be purposefully tailored to family farms, which constitute the backbone of the agricultural sector, alongside large-scale agricultural enterprises that drive regional production. To maximize the impact of these scientific breakthroughs, the generated knowledge must not remain confined to academic circles; it must be effectively transferred to the field. In this regard, agricultural advisory and extension services play a crucial role in bridging the gap between scientific theory and practical implementation.

This volume of the Proceedings brings together a curated selection of significant scientific research dedicated to addressing these vital issues in food production. The findings presented herein offer valuable insights and practical solutions aimed at fostering a more sustainable, resilient, and competitive agricultural sector.

We express our gratitude to the authors for their valuable contributions and to the reviewers for ensuring the high scientific quality of this publication. We trust that this volume will serve as an inspiring and practical resource for researchers, advisory experts, and agricultural producers alike.

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Variability of red clover seed yield and quality on acidic soil

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Abstract

On poorer quality soils and in mountainous areas, red clover is an important forage crop for animal feed production. Knowing the production potential, adaptability and stability of a variety in a particular location is of utmost importance for the success of production in the future. Soil quality, weather conditions during the second mowing (the mowing in which seed production is most often carried out) and the lifespan of red clover have a major impact on seed yield. The aim of the research in this work was to determine the production potential of the yield and quality of red clover seeds on acid reaction soil, to select the best varieties because they can be expected to give high forage yields. The selection of the best varieties is also important for the production of animal feed, if a variety has a high seed yield, it can be expected that it will also have high forage yields. Research was carried out on 10 varieties of red clover in two years in the area of the Mrkonjić Grad municipality. For all tested traits, the factors of variety and year had a statistically significant influence. The highest average seed yield was achieved by the Marina variety, the highest total germination and the lowest proportion of hard seeds by the Kolubara variety. The Viva variety had the highest 1000-kernel weight and high germination. Statistically significantly higher seed yield and 1000-kernel weight were obtained in the first year of testing, and germination and proportion of hard seeds in the second year of testing. At this location, red clover seed production should be carried out in the second mowing of the second year of life, due to low seed yields, the second mowing in the third year should be used for fodder production.

Keywords: red clover, variety, yield, seed quality

INTRODUCTION

Along with alfalfa, red clover (*Trifolium pratense* L.) is one of the most important forage legumes. Red clover requires more water but can be grown on soils with a lower pH value than alfalfa. The provision of soil moisture has a positive effect on red clover (Wang et al. 2010). According to the data of Lüscher et al. (2014) red clover is poorly resistant to drought, its cultivation is possible on soil with a pH of 4.5-8.5. Due to the possibility of cultivation in hilly and mountainous areas and on soils with poor chemical composition, red clover is an important plant species for the production of bulk fodder (Radović et al., 2010). Red clover in combination with English ryegrass and meadow fescue is suitable for establishing pastures, in mixtures for mowing it achieves high yields with cattail and Italian ryegrass (Cnops et al., 2010).

In commercial practice, less than 50% of the potential yield is usually realized (Wang et al., 2010). The yield and quality of seeded red clover can be conditioned by weather conditions during the production cycle, in a year with more precipitation, seeds with a greater mass of 1000 seeds but less germination are usually obtained (Petković et al., 2017). The average yield of red clover seeds in the second year of

life was 356 kg ha⁻¹ and in the third year 117 kg ha⁻¹ (Vasiljević et al. 2023). Tucak et al. (2016) obtained an average red clover seed yield of 172.2 kg ha⁻¹ in their trials. Red clover seed yield of less than 150 kg ha⁻¹ was obtained (Liatukas and Bukauskaitė., 2012; Nedělník et al., 2016). Lakić and Vojin (2010) obtained a higher average two-year red clover seed yield of 265 kg ha⁻¹ in Banja Luka. In the Republic of Serbia, the seed yield of red clover above 600 kg ha⁻¹ was achieved (Vasiljević et al. 2010).

Based on two-year tests, the mass of 1000 red clover seeds, depending on the genotype, was determined to be 1.41-1.75 g (Petković et al., 2017). Tetraploid varieties of red clover have a higher mass of 1000 seeds compared to diploid ones (Zelinska et al., 2012). By measuring the mass of 1,000 seeds of four varieties of red clover, it was determined that it ranged from 1.712 to 1.879 g (Atis et al., 2011).

Seed germination is an important indicator of seed quality. By examining the germination of red clover seeds, Przybylska et al. (2021) determined that it was on average 90.5-92.3%. Based on two-year analyzes of red clover seed quality, the determined average energy of seed germination was 56.0% and total germination was 90.0% (Petković et al., 2017). In the results presented by Veljević et al. (2016) the germination energy of red clover seeds ranged from 52.0% in the variety Sana to 70.0% in the variety K-17. The lowest total germination rate was found in the variety Sana, 56.0%, and the highest in the variety K-39 - 75.0%. By testing the germination of red clover seeds, the average germination energy was determined to be 56.5% in the first and 60.9% in the second year of the test (Vasiljević et al., 2023). The share of hard seeds in the total red clover seed yield decreases with the length of seed storage (Tomić et al., 2020a; Stanisavljević et al., 2021). When examining the quality of the seeds of the second cut, germination ranged from 0% to 75.25%. The genotype with the highest germination had the lowest share of hard seeds, 12.25% (Petrauskas et al., 2020). The share of hard seeds in the range of 19% - 38% was determined by the authors Veljević et al. (2016). Vasiljević et al. (2023) obtained a lower proportion of hard seeds in red clover seeds, 10.4 % in the first and 3.6 % in the second year of testing.

MATERIAL AND METHODS

The field trial was carried out on the territory of the municipality of Mrkonjić Grad, in the village of Gornji Šehovci, 748 m above sea level). The size of the basic plot was 5 m² (5x1), the distance between rows was 20 cm. The sowing rate was 17 kg ha⁻¹. Sowing was done by hand at a depth of 1.5-2 cm, with five rows in the plot. The distance between repetitions was 1 m. Sowing was done in the spring of 2017. The second in the second (2018) and the second cutting in the third (2019) year of the red clover's lifespan were used for research in this work. Half of the plot (2.5 m²) was used for the study of seed yield, and ½ of the plot was used for the study of green mass yield, which will be presented in another paper. 10 varieties of red clover were included in the tests, which are designated in this paper: Kolubara-1, Dajana-2, Nike-3, Osiris-4, Marina-5, K17-6, Zoja-7, Rozeta-8, Viva-9 and Una-10.

Before setting up the field trial, a chemical analysis of soil fertility was performed, where the reaction of the soil (pH) in H₂O and KCl, humus (%), phosphorus content (mg P₂O₅/100 g soil), potassium content (mg K₂O/100 g soil) was determined. Analyzes of soil fertility were done in the soil fertility control laboratory at the Faculty of Agriculture in Banja Luka. Meteorological data on mean monthly temperatures (°C) and precipitation amounts (mm) were taken from the Hydrometeorological Institute of the Republic of Srpska, from the nearest measuring station to the location of the field tests. The second mowing in the years of the study was carried out on the day of mowing the previous mowing with 200 kg ha⁻¹ NPK15:15:15. Chemical weed control was carried out after mowing the first mowing with the preparation Pulsar 40, using the preparation 1.0 l ha⁻¹.

The seed harvest of all varieties was carried out in one day. The seed yield was determined by measuring after harvest and processing, on an electronic scale. The obtained data were converted per unit area (hectare). In laboratory tests in the seed testing laboratory at the Faculty of Agriculture in Banja Luka, the mass of 1000 seeds (g), germination energy (%), total seed germination (%) and the proportion of hard seeds (%) were determined. The analyses were carried out using standard methods prescribed by the current Regulation (Regulation on the quality of agricultural plant seeds, Official Gazette of the Republic of Srpska No. 30/98) at the time of the implementation of these tests.

The results of biometric measurements were processed with PC applications for Windows: Statistical Package for Social Sciences, StatSoftStatistica and Excel. The results of the studied properties were processed by analysis of variance (ANOVA). Factors were year and variety. Duncan's Multiple Range Test (DMRT) was used to determine the significance of the differences between the average values and their ranking for the significance level of $\alpha=0.05$.



Figure 1. Experimental plots

Agroecological conditions

Soil samples for chemical analysis were taken from a depth of 0-30 cm. The results of laboratory analyses for humus, phosphorus, potassium content and soil pH are presented in Table 1.

Table 1. Chemical analysis of soil

Reaction (pH) in		Hummus %	P ₂ O ₅ mg/100g	K ₂ O mg/100g
H ₂ O	KCl			
5.92	4.27	6.0	1.0	24.7

The results of the soil chemical analysis showed that the soil on the sample plot had an acidic reaction, well supplied with humus, the phosphorus content was low and well supplied with potassium.

Table 2. Total monthly and annual precipitation sums (mm) and mean monthly and annual air temperatures (°C) in the period 2018 and 2019 for the location Mrkonjic Grad (Republican Hydrometeorological Institute)

	Month												Total/ average
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
P18	102.3	136.2	147.1	38.2	147.9	206.4	67.2	114.2	53.3	35.1	67.3	108.6	1223.8
P19	122.1	48.7	61.5	93.6	182.7	113.9	96.5	101.3	95.7	23.4	144.6	87.7	1171.7
T18	3.8	-1.4	3.4	13.6	15.9	17.4	19.2	20.0	15.1	12.3	6.7	0.9	10.6
T19	-1.5	3.0	7.1	10.0	11.0	20.1	20.2	20.8	15.1	11.9	9.7	3.6	10.9

P18- monthly and annual sum of precipitation for 2018; P19 - monthly and annual sum of precipitation for 2019

T18 - average monthly and annual temperature for 2018; T19 - average monthly and annual temperature for 2019

The first year was characterized by a lower mean annual temperature by 0.3 °C and a higher annual precipitation of 52.1 mm (Table 2) compared to the second year. In the period June-August 2018 (the vegetation period of the second mowing), a higher amount of precipitation and lower mean monthly air temperatures were recorded compared to the same period in the second year of testing (Table 2). In both years, weather conditions were favorable for red clover seed production.

RESULTS AND DISCUSSION

Analysis of variance (Table 3) for the investigated traits of 10 red clover cultivars revealed a statistically significant influence of the investigated factors. The interaction year x cultivar also had a significant influence on seed yield.

Table 3. Analysis of variance of seed yield, 1000-kernel weight, seed germination energy, total seed germination and proportion of hard seeds of 10 red clover varieties in 2018 and 2019

	SS	df	MS	F
Grain yield				
Growing season(a)	292820	1	292820	2998.413**
Variety (b)	16995.7	9	1888.41	19.34**
Growing season*variety (axb)	5058	9	562.0	5.76**
Error	5859.5	60	97.66	
Total	3041002	80		
Kernel weight				
Growing season(a)	0.13	1	0.13	21.63**
Variety (b)	0.57	9	0.06	10.68**
Growing season*variety (axb)	0.09	9	0.01	1.68 ^{ns}
Error	0.36	60	0.01	
Total	220.57	80		
Seed germination energy				
Growing season(a)	952.2	1	952.20	38.16**
Variety (b)	1920.3	9	213.37	8.55**
Growing season*variety (axb)	226.3	9	25.14	1.01 ^{ns}
Error	1497.0	60	24.95	
Total	264972.0	80		
Total seed germination				
Growing season(a)	2215.51	1	2215.51	141.08**
Variety (b)	658.01	9	73.11	4.66**
Growing season*variety (axb)	260.61	9	28.96	1.84 ^{ns}
Error	942.25	60	15.70	
Total	567381.0	80		
Proportion of hard seeds				
Growing season(a)	530.45	1	530.45	27.09**
Variety(b)	1789.70	9	198.86	10.15**
Growing season*variety (axb)	255.05	9	28.34	1.45 ^{ns}
Error	1175.00	60	19.583	
Total	53152.00	80		

The obtained average values of the tested traits by varieties were statistically significantly different (Table 4). In the first year of testing, a statistically significantly higher seed yield and 1000-kernel weight were achieved, and in the second year, higher germination and hard seed content (Table 4).

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Table 4. Average values of seed yield (kg ha⁻¹), 1000 seed weight (g), seed germination energy (%), total seed germination (%) and proportion of hard seeds (%) of 10 tested red clover varieties in 2018 and 2019

Variety	Year		Average by variety
	2018	2019	
Seed yield			
1	226.8	121.3	174.0 ^e
2	256.0	118.8	187.4 ^{cd}
3	191.8	105.3	148.5 ^f
4	254.3	116.8	185.5 ^{cd}
5	264.0	140.8	202.4 ^a
6	246.0	135.8	190.9 ^{bc}
7	267.5	132.5	200.0 ^{ab}
8	259.5	126.5	193.0 ^{abc}
9	242.5	114.5	178.5 ^{de}
10	240.8	127.0	183.9 ^{cde}
Average per Year	244.9 ^a	123.9 ^b	184.4
Kernel weight			
1	1.65	1.55	1.60 ^d
2	1.80	1.71	1.75 ^{ab}
3	1.81	1.67	1.74 ^{abc}
4	1.55	1.47	1.51 ^e
5	1.71	1.65	1.68 ^{abcd}
6	1.47	1.57	1.52 ^e
7	1.71	1.62	1.66 ^{cd}
8	1.69	1.64	1.67 ^{bcd}
9	1.82	1.69	1.76 ^a
10	1.75	1.60	1.68 ^{abcd}
Average per Year	1.70 ^a	1.62 ^b	1.66
Energy of germination seeds			
1	57.8	59.0	58.4 ^{bcd}
2	59.0	63.0	61.0 ^b
3	46.5	50.8	48.6 ^e
4	50.0	57.0	53.5 ^{cde}
5	50.8	58.5	54.6 ^{cd}
6	52.8	65.3	59.0 ^{bc}
7	55.0	66.5	60.8 ^b
8	64.5	69.0	66.8 ^a
9	49.5	56.3	52.9 ^{de}
10	50.3	59.8	55.0 ^{cd}
Average per Year	53.6 ^b	60.5 ^a	57.1
Total seed germination			
1	83.0	91.0	87.0 ^a
2	83.5	90.0	86.8 ^a
3	83.3	89.3	86.3 ^a
4	71.5	88.3	79.9 ^{bc}
5	74.3	89.0	81.6 ^{bc}
6	72.8	86.3	79.5 ^c
7	77.5	90.8	84.1 ^{ab}
8	82.3	89.8	86.0 ^a
9	82.5	90.8	86.6 ^a
10	76.0	86.8	81.4 ^{bc}
Average per Year	78.7 ^b	89.2 ^a	83.9
Proportion of hard seeds			
1	12.0	23.0	17.5 ^f
2	21.5	30.3	25.9 ^{bc}
3	21.5	23.3	22.4 ^{cde}
4	29.0	31.0	30.0 ^{ab}
5	15.3	23.8	19.5 ^{ef}
6	22.0	26.8	24.4 ^{cd}
7	22.0	28.0	25.0 ^{cd}
8	30.8	31.8	31.3 ^a
9	31.8	32.0	31.9 ^a
10	17.0	24.5	20.8 ^{def}
Average per Year	22.3 ^b	27.4 ^a	24.9

Variety 3 had the lowest germination energy and grain yield, the grain yield of variety 3 was statistically significantly lower compared to all varieties. The seeds with the highest total germination and the lowest proportion of hard seeds were from variety 1, and the highest 1000-kernel weight was from variety 9. The highest grain yield was achieved by variety 5, but this variety had low seed germination energy. Variety 8 had significantly higher seed germination energy compared to other varieties.

The average red clover seed yields achieved in this study ranged from 148.5 kg ha⁻¹ for variety 3 to 202.4 kg ha⁻¹ for variety 5, while the average two-year yield of all varieties was 184.4 kg ha⁻¹ (Table 4). In research conducted in the Republic of Croatia, the highest red clover seed yield of 272.71 kg ha⁻¹ was obtained with the Viva variety. The lowest seed yield of 68.13 kg ha⁻¹ was obtained with the Titus variety. The average seed yield of all populations and varieties included in the author's research in the Republic of Croatia was 172.2 kg ha⁻¹ (Tucak et al., 2016). The average red clover seed yield in our research in 2018 was 244.9 kg ha⁻¹ (Table 4), similar data for the achieved seed yield in the second year of red clover life were obtained by Petković et al. (2017), in whose studies conducted in the mountainous area of the city of Banja Luka, the average red clover seed yield in the second year of life was 223.0 kg ha⁻¹. The red clover seed yield is quite variable and may also depend on the lifespan of the red clover, with the highest yields being achieved in the second year of life (Petković, et. al., 2017; Tomić et al., 2020b). The authors Stevović et al. (2012), by testing four red clover varieties, achieved red clover seed yields in a larger interval from 169 to 310 kg ha⁻¹.

In relation to the achieved average red clover seed yield (Table 4), a higher average seed yield of 265 kg ha⁻¹ in Bosnia and Herzegovina was obtained by Lakić and Vojin (2010), as well as Vasiljević et al. (2010) in the Republic of Serbia. Lower seed yields compared to the data from these studies were shown by Liutukas and Bukauskaitė (2012), who, in four-year trials of 58 diploid varieties in Lithuanian conditions, achieved an average seed yield of 110.4 kg ha⁻¹, and the average seed yield of 35 tetraploid varieties was 94.9 kg ha⁻¹.

The presented results of our research show that in the first year, a higher average seed yield and a higher average weight of 1000 seeds were achieved. The first year of research is characterized by higher precipitation and lower average air temperature (Table 2), higher seed yields and a higher weight of 1000 seeds in a year with higher precipitation were obtained by the authors Petković et al. (2017). The average weight of 1000 seeds in this work was 1.66 g. The variation of the weight of 1000 seeds by variety (Table 4) ranged from 1.51 g for variety 4 to 1.76 g for variety 9. The weight of 1000 red clover seeds in the interval 1.712-1.879 g was determined by (Atis et al., 2011); 1,144-1,544 g (Stevović et al., 2012); 1.41–1.75 g (Petković et al., 2017).

Energy and total germination represent significant parameters of seed quality, higher values of these parameters were determined in the second year (Table 4). The seed with the highest germination energy of 66.8% was found in variety 8, and the highest total germination of 87.0% was found in variety 1. Compared to our average data on total germination by year (in the first 78.7% and the second 89.2%), Przybylska et al. (2021) presented higher total germination of red clover seeds (in the first 90.5% and the second 92.3%) in their studies. In these studies, the average germination energy of red clover seeds was 57.1% and the total germination was 83.9% (Table 4). An approximate value of the germination energy of red clover seeds of 56%, but a higher total germination rate, was obtained by Petković et al. (2017). Compared to our seed germination data (Table 4), Stanisavljević et al. (2021) analyzed the quality of red clover seeds in the year of harvest and determined a higher germination energy of red clover seeds of 67%, but a lower total seed germination rate of 75%. Eight months after the harvest of red clover seeds, seed germination in the Tomić et al. (2020a) studies was 89.6%. Our average total seed germination rate is lower compared to the total germination rate shown by Bender and Tamm (2018), in the work of these authors, the germination rate of red clover seeds was above 95.0%.

The obtained data for the proportion of hard seeds showed that a statistically significantly higher proportion of hard seeds was obtained, 27.4%, in the second year compared to the proportion of hard seeds of 22.3%, which was determined in the first year of the study (Table 4). In this work, the average two-year proportion of hard seeds was 24.9%. The proportion of hard seeds decreases with the length of seed storage (Tomić et al., 2020a; Stanisavljević et al., 2021). In the studies of Tomić et al. (2020a), the proportion of hard seeds was the lowest 20 months after harvest, 0.67%, and the highest one month

after harvest, 52.8%. Przybylska et al. (2021) obtained an average proportion of hard seeds that ranged from 2.6% in seeds from the first year to 26.2% in seeds obtained from the second year of the study. Our lowest proportion of hard seeds of 17.5% was determined in variety 1, while the highest, 31.9, was in variety 9. Similar data on the proportion of hard seeds of red clover in the interval 19% - 38% were determined by the authors Veljević et al. (2016).

CONCLUSION

Vegetation seasons and red clover varieties have a strong influence on the tested red clover traits. At the location of the tests, the production of quality red clover seeds can be realized in the second mowing of the second and third years of life. However, seed production should be carried out in the second year of life, due to the relatively low yields achieved of 123.9 kg ha⁻¹ and the biological properties of red clover that in the second mowing of the third year of life it gives low seed yields, it is recommended that this mowing in that year of life is used for fodder production. Based on the achieved results, varieties 5, 7 and 8 are recommended for wider use in seed production. It can be concluded that the other seven varieties also achieved good results and represent good material, depending on what the goal of production in the future period is: seeds with a higher mass of 1000 seeds, germination or a lower proportion of hard seeds, each of the seven had good results for a certain trait.

REFERENCES

- Atis, A., Atak, M., Can, E., & Mavi, K. (2011). Seed Coat Clover Effects on Seed Quality and Salt Tolerance of Red Clover (*Trifolium pratense*). *International Journal of Agriculture & Biology*, 1(3), 363-368.
- Bender, A., & Tamm, S. (2018). Seed yield of tetraploid red clover as influenced by cover crop management. *Zemdirbyste-Agriculture*, 105(2), 133-140. <https://doi.org/10.13080/z-a.2018.105.017>
- Cnops, G., Antje, R., Saracutu, O., Malengier, M., & Roldan-Rouz, I. (2010). Morphological and Molecular Diversity of Branching in Red Clover (*Trifolium pratense*). In: Huyghe, C. (ed.), *Proceedings of the Conference of the Eucarpia fodder and Amenity Species Section: Sustainable use of genetic diversity in forage and turf breeding*. Dordrecht, Heidelberg, London, New York, pp. 73-77.
- Lakić, Ž., & Vojin, S. (2010). Variability of agronomic traits of red clover genotypes (*Trifolium pratense* L.). *Biotechnology in Animal Husbandry*, 26 (Spec. Issue 2), 35-40.
- Liatukas, Ž., & Bukauskaitė, J. (2012). Differences in Yield of Diploid and Tetraploid Red Clover in Lithuania. *Proceedings of the Latvian Academy of Sciences. Section B*, 66(4/5), 163-167. <https://doi.org/10.2478/v10046-012-0023-y>
- Nedělník, J., Knotova, D., Pelikan, J., Vymyslický, T., Mihovski, T., & Naydenova, G. (2016). Comparing Yield Potential of Czech and Bulgarian Red Clover (*Trifolium pratense* L.) Seeds. *Journal of Mountain Agriculture on the Balkans*, 19(1), 38-45.
- Petrauskaas, G., Norkevičienė, E., Stukonis, V., & Kemešytė, V. (2020). Phenotypic traits for wild red clover seed yield under drought conditions. *Czech Journal of Genetics and Plant Breeding*, 56(4), 140-149. <https://doi.org/10.17221/111/2019-CJGPB>
- Petković, B., Pržulj, N., Radić, V., & Mirosavljević, M. (2017). Comparative study of seed yield and seed quality of advanced lines and commercial varieties of red clover (*Trifolium pratense* L.). *Legume Research*, 40(6), 1066-1071. <https://doi.org/10.18805/LR-360>
- Przybylska, A., C'wintal, M., Pszczółkowski, P., & Sawicka, B. (2021). Effect of Attractants and Micronutrient Biofortification on the Yield and Quality of Red Clover (*Trifolium pratense* L.) Seeds. *Agronomy*, 11(52), 1-28. <https://doi.org/10.3390/agronomy11010152>
- Radović, J., Katić, S., Lugić, Z., & Vasiljević, S. (2010). Breeding of perennial forage legumes in Serbia, results and future directions. *Biotechnology in Animal Husbandry*, 26 (Spec. Issue), 67-79.

- Stanisavljević, R., Poštić, D., Štrbanović, R., Oro, V., Tabaković, M., Jovanović, S., & Milenković J. (2021). Variability and Correlative Interdependence of Red Clover and Italian Ryegrass Seed Quality Depending on Varieties During the Multi-Year Storage Period. *Journal on Processing and Energy in Agriculture*, 25(1), 1-6. <https://doi.org/10.5937/jpea25-30969>
- Stevović, V., Tomić, D., Đurović, D., Bokan, N., & Stanisavljević, R. (2012). Effect of boron on seed yield and components of red clover (*Trifolium pretense* L.) cultivars grown under dense planting conditions. *47th Croatia and 7 International Symposium on Agriculture*, pp. 331-334.
- Tomić, D., Stevović, V., Đurović, D., Stanisavljević, R., Madić, M., Petrović, M., Lazarević, Đ., & Knežević, J. (2020a). Seed testing of foliar-fertilised red clover crops after various periods of storage. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 48(1), 284-293. <https://doi.org/10.15835/nbha48111814>
- Tomić, D., Stevović, V., Simić, A., Đurović, D., Radanović, M., Madić, M., & Knežević, J. (2020b). Foliar fertilization with phosphorus and potassium in red clover seed production on an acidic soil. *Acta Agriculturae Serbica*, 25(49), 51-57. <https://doi.org/10.5937/AASer2049051T>
- Tucak, M., Popović, S., Čupić, T., Krizmanić, G., Španić, V., Meglič V., & Radović, J. (2016). Assessment of red clover (*Trifolium pratense* L.) productivity in environmental stress. *Poljoprivreda*, 22(2), 3-9. <https://doi.org/10.18047/poljo.22.2.1>
- Vasiljevic, S., Pataki, I., Šurlan-Momirovic, G., & Živanovic, T. (2005). Production potential and persistence of red clover varieties. *Grassland Science in Europe*, 10, 577-580.
- Vasiljević, S., Karagić, Đ., Mihailović, V., Pataki, I., Milošević, B., & Pejić, B. (2010). Effect of sowing method and seeding rate on yield components and seed yields in red clover (*Trifolium pratense* L.). *Biotechnology in Animal Husbandry*, 26 (Spec. Issue), 159-165.
- Velijević, N., Simić, A., Vučković, S., Đukanović, L., Poštić, D., Štrbanović, R., & Stanisavljević, R. (2016). Variability of dormancy, seed germination and seedling vigor of cultivars of red clover and Italian ryegrass cultivars. *Proceedings XXI Consultation on Biotechnology with international participation*, 1, 73-80.
- Vasiljević, S., Nikolić, Z., Katanski, S., Mamlić, Z., Đukić, V., Uhlarik, A., Dolapčev Rakić, A., & Balać, M. (2023). The effect of cutting management on seed yield, seed yield components, and seed quality of red clover (*Trifolium pratense* L.). *Agro-knowledge Journal*, 24(1), 1-12. <https://doi.org/10.7251/AGREN2301001V>
- Wang, J., Drayton, C. M., & George, J. (2010). Identification of genetic factors influencing salt stress tolerance in white clover (*Trifolium repens* L.) by QTL analysis. *Theoretical and Applied Genetics*, 120, 607-619. <https://doi.org/10.1007/s00122-009-1179-y>
- Zielinska, M., Zapotoczny, P., Bialobrzewski, I., Zuk-Golaszewska, K., & Markowski, M. (2012). Engineering properties of red clover (*Trifolium pretense* L.) seeds. *Industrial Crops and Products*, 37, 69-75. <https://doi.org/10.1016/j.indcrop.2011.12.002>

Variation of weight of spike in wheat varieties

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Abstract

The weight of the spike serves as a measure of the wheat seed yield. This study aimed to assess the variation in the weight of the spike among 50 genetically diverse wheat varieties. The experiment was set up as a randomized block design consisting of three replications during the 2016/17 growing season. The seeds were planted at a distance of 0.10 m in rows that were 1.0 m long, with 0.2 m between each row. Sixty fully mature plants (20 plants per replication) were collected and utilized to assess the weight of the spike. The variance analysis was carried out using MSTAT C (version 5.0). The hierarchical method of Euclidean distance was utilized to analyze similarities among wheat varieties. The results obtained showed considerable differences in the average values of the spike weight among the wheat varieties in the experimental year. The lowest weight of the spike was in the Kosmajka and Šumadija varieties (2.79 g), whereas the Milica variety had the highest spike weight (4.45 g). The average weight of the spike for all 50 wheat varieties was 3.51 g. The coefficient of variation values ranged from 10.4% for Danica to 20.5% for the Kremna variety. Similarity was illustrated in a dendrogram containing five clusters. The similarity was depicted in a dendrogram containing five groups of mutually similar varieties. The prominent cluster contained different numbers and compositions of varieties with the highest degree of similarity.

Keywords: wheat, spike weight, variety, similarity

INTRODUCTION

The weight of spike is a quantitative component of wheat phenotypic variability whose heredity is governed by a minor gene system that, in conjunction with environmental factors, affects its variation (Álvaro et al., 2008; Knezevic et al., 2014; Xie et al., 2018). The impact of genetic and environmental influences, along with their interaction on this trait, is considerable (Kraljević-Balalić and Knežević, 1993; Knežević et al. 2019; Matković Stojšin et al., 2015; Sugár et al., 2016; Kondić et al., 2017a; Cohen et al., 2020). Abiotic factors (soil fertility, crop nutrition, temperature, and soil moisture) directly influence the development of productive shoots (Valerio et al., 2009; Xie et al., 2016; Kondić et al., 2017b; Zečević et al., 2021; Matković Stojšin et al., 2022), with their quantity being contingent on soil type and linked to changes in spike weight and the implementation of improvement methods (Dimitrijević et al., 2006; 2011; Banjac et al., 2009; 2015; Petrović et al., 2016; Madić et al., 2019; Marković et al., 2020). The weight of spike affects yield per plant and indicates total seed yield in correlation with other properties of spike, seed properties, productive tillers, height of stem, green leaf area, root absorption capacity (Baillot et al., 2018; Würschum et al., 2018; Cao et al., 2020). To achieve the genetic potential for a high and consistent yield along with its components, it is essential to understand the genotype's life

cycle, the specific agroecological conditions (Cohen et al., 2020; Knežević et al., 2020; Šumaruna et al., 2022), and to choose wheat genotypes that have a favorable gene combination for enhanced efficiency in absorbing water, minerals, and light, which is critical for optimal organogenesis and the plant's life cycle (Knezevic et al., 2007; Petrović et al. 2012; Marković et al., 2020). The enhancement of these traits is positively associated with seed filling, size and weight, spike weight, spike length, and spikelet number per spike (Zečević et al., 1997; 2016; Saeidi and Abdoli, 2015). Enhancing the ability of the spikes to absorb assimilates is crucial, especially during the post-flowering phase when photosynthetically significant leaves start to senesce quickly (Sreenivasulu and Schnurbusch, 2012).

The aim of this research is to determine the variability of spike weight in winter wheat varieties grown in sparse sowing under same agroecological conditions.

MATERIALS AND METHODS

The study comprised 50 genetically divergent wheat varieties developed in various breeding centers. The experiment was conducted in field conditions in Kraljevo, Serbia, following a random block system design with three replications throughout the 2016/17 growing season. Sowing occurred by hand in rows of 1.0 m, with a spacing of 0.20 m between the rows and 0.10 m between the plants in each row. Plant samples for spike weight analysis were collected at the full maturity stage. In total, 60 plants (20 plants across 3 replicates) were examined for each variety. The obtained data were analyzed through monofactorial analysis of variance utilizing the MSTAT C version 5.0 software, with significant differences assessed using F-test values and verified through LSD test value at 0.05 and 0.01. The hierarchical method of Euclidean distance was used to determine the similarity among the analyzed wheat.

Weather conditions

The mean temperature was 8.74 °C, and the cumulative precipitation was 523 mm during the experimental growing season (2016/17), exceeding the averages from the previous 10 years, where the mean temperature was 8.50 °C and the precipitation was 417.8 mm (Table 1). Over the course of two months (October-November), the levels of rainfall and average temperature readings were comparable, which benefits seed germination and plant growth. From February to April, the precipitation in the experimental year (174.0 mm) surpassed that of the 10-year average (116.0 mm), showing a more advantageous distribution for plant growth in the experimental year compared to the long-term period (Table 1).

Table 1. Average monthly temperature and total monthly precipitation in Kraljevo

	Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Avg	Total
Temperature (°C)	2016/17	10.6	6.8	0.0	-4.7	5.2	10.8	11.1	16.8	22.1	8.74	
	2000-2010	11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	8.50	
Precipitation (mm)	2016/17	84.1	77.6	9.4	22.0	35.0	57.0	82.0	100.0	56.0		523.0
	2000-2010	61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3		417.8

RESULTS AND DISCUSSION

The research showed that the weight of spikes in the studied wheat varieties varied from the lowest 2.79 g in the Kosmajka and Šumadija varieties to the highest spike weight of 4.45 g in the Milica variety, with an average value of 3.51 g for all 50 varieties (Table 2).

Table 2. Variability of weight of spike in wheat varieties

	Cultivars	$\bar{x} \pm s_x$	S	CV (%)		Cultivars	$\bar{x} \pm s_x$	S	CV (%)
1	Evropa 90	3.42±0.08	0.59	17.2	26	Jarebica	3.37±0.08	0.59	17.3
2	Dejana	3.79±0.08	0.61	15.2	27	Fortuna	2.92±0.06	0.50	17.0
3	Sila	3.65±0.08	0.59	15.9	28	Sasanka	3.38±0.06	0.48	14.0
4	Omega	3.77±0.08	0.58	15.2	29	Danica	3.83±0.05	0.40	10.4
5	Lasta	3.66±0.06	0.47	12.9	30	Somborka	3.65±0.08	0.64	17.3
6	Milica	4.45±0.06	0.49	11.0	31	Kremna	3.54±0.09	0.73	20.5
7	Parizanka	3.47±0.05	0.42	12.1	32	KG-75	3.92±0.08	0.61	15.5
8	Pobeda	3.41±0.09	0.67	19.4	33	Šumadija	3.86±0.07	0.56	14.4
9	Dična	3.73±0.06	0.49	13.3	34	Levčanka	3.87±0.07	0.56	15.2
10	NS Rana 5	3.64±0.09	0.67	18.3	35	Oplenka	4.09±0.06	0.50	12.0
11	Alfa	3.29±0.08	0.59	17.7	36	Gruža	3.67±0.08	0.62	16.7
12	Rodna	3.54±0.08	0.59	16.8	37	Gružanka	2.97±0.05	0.41	13.6
13	Balkan	3.31±0.07	0.50	15.0	38	KG-58	3.45±0.06	0.49	14.4
14	Rana Niska	2.87±0.07	0.50	17.4	39	KG-56	3.63±0.07	0.55	15.1
15	Proteinka	3.90±0.08	0.65	16.5	40	Orašanka	3.53±0.07	0.53	15.0
16	Stepa	3.54±0.06	0.44	12.3	41	KG-78	3.49±0.07	0.57	16.3
17	NSR-2	3.35±0.08	0.58	17.2	42	Ravanica	3.69±0.07	0.56	15.0
18	Prima	3.25±0.07	0.60	17.2	43	Lepenica	3.50±0.08	0.65	18.4
19	Sloga	3.58±0.09	0.74	20.4	44	Jasenica	3.69±0.08	0.65	17.5
20	Agrounija	3.33±0.08	0.60	17.7	45	Zastava	3.30±0.08	0.60	18.0
21	Zadruga	3.69±0.07	0.57	15.2	46	Kosmajka	2.79±0.06	0.46	16.4
22	Tera	3.28±0.05	0.39	12.0	47	Šumadija	2.79±0.06	0.46	16.4
23	Kompas	3.60±0.07	0.56	15.3	48	Morava	3.22±0.08	0.61	18.8
24	Tanjugovka	3.50±0.07	0.52	14.4	49	KG 56 S	3.34±0.08	0.60	17.9
25	Jugoslavija	3.67±0.06	0.45	12.0	50	Ljubičevka	3.50±0.07	0.50	14.2
	Average						3.51±0.07	0.55	15.7

The variation of spikes weight of Serbian wheat in earlier studies was comparable, ranging from 3.00 g in the Zadruga variety to 4.23 g in the Gruža variety (Knežević et al., 2010), from 3.28 g in KG-3419-3/97 to 4.23 g in KG-3625/97 (Knežević et al., 2011), from 3.30 g for the Zadruga variety to 4.49 g for the Milica variety, from 3.20 g for the Alfa variety to 3.92 g for the Oplenka variety (Knežević et al., 2019), and from 2.74 g for the Tera variety to 4.51 g for the Milica variety (Urošević et al., 2025). Variations in the weight of spikes in wheat varieties are determined by genotypic specificity, growing conditions, and the effect of genotype/environment interaction that influences physiological and biochemical processes during the stages of organ growth and development.

Comparable outcomes for the weight of spikes on chernozem soil ranged from 1.96 g in the Cheyene variety to 3.80 g in the Avalon variety (Šumaruna et al., 2022). In a different study performed over two successive years, 2014/15 and 2015/16, the spike weight ranged from 2.6 g and 2.5 g in the Pesma variety to 3.8 g and 3.0 g in the Renesansa variety on chernozem, while it varied from 1.1 g for the Pesma variety to 1.2 g for the Renesansa variety on solonetz soil (Petrović et al., 2016). In comparable

research involving 27 wheat varieties on chernozem soil, the average spike weight ranged from 1.6 g in the Šumadija variety to 2.9 g in the Morava variety, whereas on solonetz soil, the spike weight varied from 1.1 g in the Banatka variety to 2.0 g in the Kragujevčanka 58 variety (Matković Stojšin et al., 2022). These studies showed that soil characteristics affect the weight value of the spike.

The average recorded weight of spikes (1.4-1.5 g) was notably lower in 12 wheat varieties examined over three growing seasons on the solonetz, where the minimum value was 0.5 g and the maximum reached 4.0 g (Banjac et al., 2009; Banjac, 2015). The analysis of the spike weight revealed variations among the studied wheat varieties. This suggests that the changes in morphophysiological traits rely on the genotypic structure and the adaptive response to environmental influences, along with the impact of agroecological and climatic conditions (Liu et al., 2009; Zečević et al., 2021).

The standard deviation and the coefficient of variation show high variability in the weight of spike among the studied wheat varieties. The greatest variability for the weight of the primary spike in the 2016/17 growing season was observed in the Kremna variety (CV = 20.5%), while the lowest was in the Danica variety (CV = 10.4%). On average, for all 50 tested varieties, the coefficient of variation for the trait of spike weight was CV = 15.7%. In a previous study of the same 50 varieties in different growing seasons, the coefficient of variation for the weight of the spike ranged from 11.0% in the Orašanka variety to 24.6% in the Lepenica variety, with an average value of 16.5% for all 50 varieties.

The analysis of variance revealed that the differences between the varieties for the trait weight of spike were significant ($p < 0.01$). The revealed significant differences in the average values of weight of spike, indicating genetic divergence of varieties (Table 3).

Table 3. Analysis of variance for weight of spike in wheat

Source of variance	Vegetation season 2016/17					
	df	SS	MS	F	Lsd _{0.05}	Lsd _{0.01}
Genotypes (G)	49	14.853	0.303	11.791**	0.364	0.482
Repetitions (R)	2	2.520	0.026			
Total	149	17.373				

Based on the expressed differences between varieties according to primary spike weight, the varieties can be grouped according to the degree of mutual similarity for class weight. Based on hierarchical cluster analysis in the vegetation season (2016/17), five clusters were identified with different numbers of mutually similar varieties according to weight of spike. In addition to the six clusters, with a different number of similar varieties according to the weight of spike (Fig. 1).

In the first cluster there are 12 varieties that are similar to each other according to the weight of the primary cluster, in the second cluster there are 10 varieties, in the third cluster 13 varieties, in the fourth cluster there are eight varieties, in the fifth cluster two varieties, in the sixth cluster there are five similar varieties.

The formed clusters have different levels of similarity of varieties according to the weight of the primary spike. The greatest similarity, or the smallest distance (distance), was between the first and second clusters of varieties. At a slightly greater distance is the similarity between the fourth and fifth clusters of varieties. The third cluster of varieties had the greatest similarity with the first and second clusters of varieties, and together this cluster had similarity with the formed cluster of the fourth and fifth clusters of varieties, but at a greater distance. At the greatest distance according to the similarity of the weight of the primary spike was the sixth cluster with all five previously combined clusters of varieties (Fig. 1).

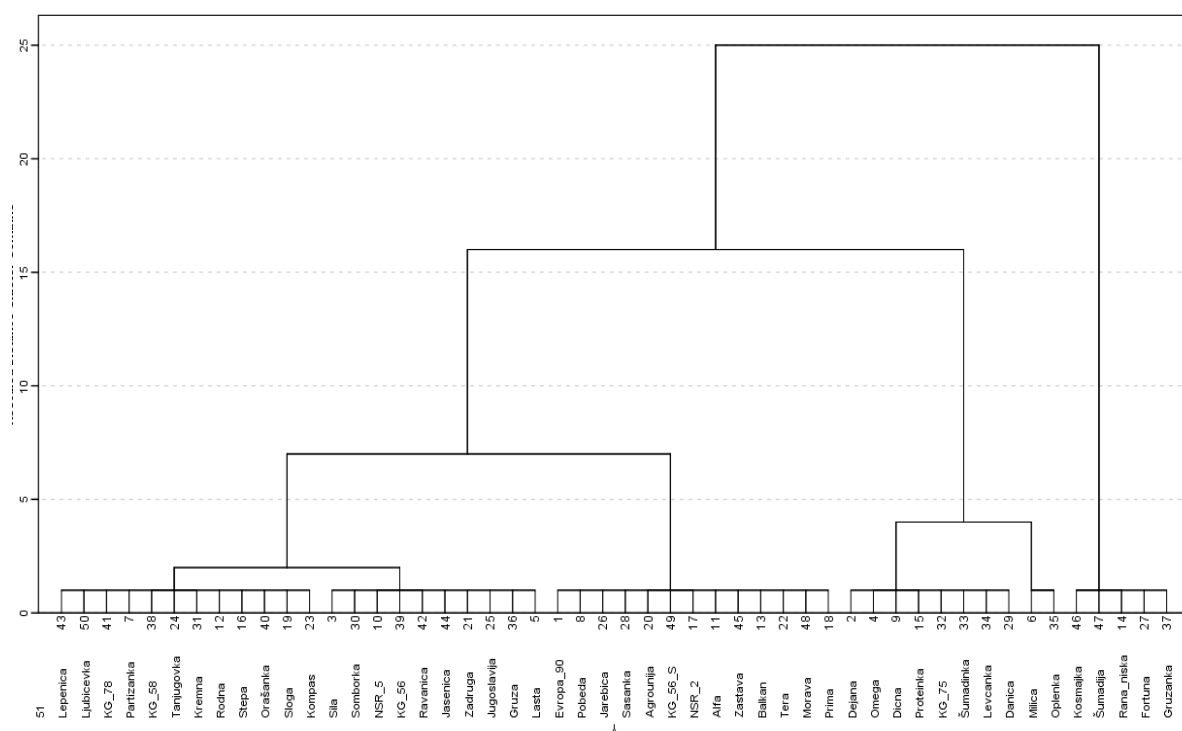


Figure 1. Similarity of wheat genotypes according to weight of primary spike in 2016/17

In previous studies of these 50 varieties in different climatic conditions, hierarchical cluster analysis showed five clusters of similar varieties according to the weight of the clusters. In addition to the five clusters, one variety, Terra, was also distinguished, which had similarities with the first cluster (Urošević et al., 2025). It is noted that the number and varieties in the clusters are different compared to the grouped varieties in these studies.

The major factors contributing to the rise in spike weight and yield include soil moisture, nutrient minerals, the availability of minerals for uptake, temperature, light, the photosynthetic process, and the recycling and movement of organic materials (Álvaro et al., 2008; Jelić et al., 2017; Sugár et al., 2016). A primary focus for a plant breeder is the ongoing enhancement of the best genotypes to boost their yield potential, either directly or indirectly, by optimizing different elements that lead to high yields (Arya et al., 2017; Zečević et al., 2021).

CONCLUSIONS

In this study, significant differences among wheat genotypes were determined considering the weight of the primary spike. The variation of the weight of the spike among varieties in the 2016/17 vegetation season indicates the significant genotypes' effect to the expression of the tested trait. The highest weight of the spike was found in the Milica variety (4.45 g), while the least was found in Kosmajka and Šumadija (2.79 g). The differences between genotypes were significant and highly significant for the weight of the primary spike.

The studied varieties showed mutual similarity in terms of cluster weight. Based on the cluster weight values, the varieties were grouped into six groups of varieties that had the greatest similarity to one another.

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REFERENCES

- Álvarez, F., Isidro, J., Villegas, D., García del Moral, L.F., & Royo, C. (2008): Old and modern durum wheat varieties from Italy and Spain differ in main spike components. *Field Crops Research*, 106:86-93. <https://doi.org/10.1016/j.fcr.2007.11.003>
- Arya, V.K., Singh, J., Kumar, L., Kumar, R., Kumar, P., & Chand, P. (2017): Genetic variability and diversity analysis for yield and its components in wheat (*Triticum aestivum* L.). *Indian Journal of Agricultural Research*, 51(2): 128-134. <http://doi.org/10.18805/ijare.v0iOF.7634>
- Baillot, N., Girousse, C., Allard, V., Piquet-Pissaloux, A., & Le Gouis, J. (2018): Different grain filling rates explain grain-weight differences along the wheat ear. *PLoS ONE*, 13(12): e020959. <https://doi.org/10.1371/journal.pone.0209597>
- Banjac, B., Dimitrijević, M., & Petrović, S. (2009): Variation of plant height and spike weight in wheat grown on solonetz soil. *Letopis Naučnih Radova*, 33(1):93-10.
- Banjac, B., Dimitrijević, M., Petrović, S., & Mladenov, V. (2015): Stability of yield components of bread wheat grown on different soil types. *Selekcija i semenarstvo*, 21 (2), 81-92. <https://doi.org/10.5937/SelSem1502081B>
- Cao, S., Xu, D., Hanif, M., Xia, X., & He, Z. (2020): Genetic architecture underpinning yield component traits in wheat. *Theoretical and Applied Genetics*, 133, 1811–1823. <https://doi.org/10.1007/s00122-020-03562-8>
- Cohen, I., Zandalinas, S.I., Huck, C., Fritsch, F.B., & Mittler, R. (2020): Meta-analysis of drought and heat stress combination impact on crop yield and yield components. *Physiologia Plantarum*, 171, 66–76. <https://doi.org/10.1111/pp1.13203>
- Dimitrijević, M., Petrović, S., Belić, M., & Vuković, N. (2006): Phenotypic variation of wheat spike parameters on meliorated solonetz. *Plant Breeding and Seed Production*, 1-2, 27-33.
- Dimitrijević, M., Knežević, D., Petrović, S., Zečević, V., Bošković, J., Belić, M., Pejić, B., & Banjac, B. (2011): Stability of yield components in wheat (*Triticum aestivum* L.). *Genetika*, 43(1), 29-39. <https://doi.org/10.2298/GENSR1101029D>
- Jelic, M., Kastori, R., Dugalić, G., & Knežević, D. (2008): Environmental and genetical influences on nutritional status of wheat grain. *Cereal Research Communications*, 36, 683-686.
- Knežević, D., Kovacevic, V., Paunovic, A., Madić, M., & Djukic, N. (2007): Genetic analysis of physiological efficiency of nitrogen in four wheat cultivars and their hybrids. Proceedings of 1st International Conference “Research People and Actual Tasks on Multidisciplinary Sciences” 6 – 8 June 2007, Lozenec, Bulgaria, pp. 16-20.
- Knežević, D., Branković, G., Šurlan-Momirović, G., Stamenković, S., & Knežević, J. (2010): Phenotypic variability of weight of wheat (*Triticum aestivum* L.) primary spike. *Arhiv za poljoprivredne nauke*, 71 (3), 255, 15-20.
- Knežević, D., Kovačević, V., Paunović, A., Madić, M., Đukić, N., Tanasković, S., & Jelić, M. (2011): Variability of mass of spike in wheat *Triticum aestivum* L. XVI. Conference on biotechnology, Univ. of Kragujevac, Faculty of Agric., Čačak, 4-5 March 2011. Proceedings, 16(18), 47-53.
- Knežević, D., Paunovic, A., Brankovic, G., Maklenovic, V., & Brocic, Z. (2014): Variability of mass of spike in wheat genotype *Triticum aestivum* L. in different environment. 49th Croatian and 9th International Symposium on Agriculture, 16-21 February 2014, Dubrovnik, Croatia. Proceedings, 367-371.
- Knežević, D., Paunović, A., Zečević, V., Urošević, D., Kondić, D., Mićanović, D., Živić, J., Madić, M., Djurović, V., Srdić, S., & Kovačević, V. (2019): Variability of mass of spike in winter wheat (*Triticum aestivum* L.) cultivars. XXIV Conference on biotechnology, University of Kragujevac, Faculty of Agriculture Čačak, 15-16 March 2019, Čačak, Serbia. Proceedings, 24(26), 37-44.
- Knežević, D., Laze, A., Paunović, A., Djurović, V., Đukić, N., Valjarević, D., Kondić, D., Mićanović, D., Živić, J., & Zečević, V. (2020): Approaches in cereal breeding. *Acta Agriculturae Serbica*, 25(50):179-186. <http://dx.doi.org/10.5937/AASer2050179K>

- Kraljević-Balalić, M., & Knežević, D. (1993): Genetic analysis of spike traits in breed wheat. Proceedings of 8th International Wheat Genetic Symposium, Beijing, China, 2, 861-864.
- Kondić, D., Bajić, M., Hajder, Đ., Knežević, D., & Bosančić, B. (2017a): The spike characteristics of winter wheat (*Triticum aestivum* L.) varieties in agro-ecological conditions of Banja Luka region. *Agroznanje*, 18(4), 263–274. <https://doi.org/10.7251/AGREN1704263K>
- Kondić, D., Bajić, M., Hajder, Đ., & Knežević, D. (2017b): Variability of number of spikes per unit area and grain yield effected by different sowing densities of winter wheat (*Triticum aestivum* L.). *Agroznanje*, 18(2), 131–141. <https://doi.org/10.7251/AGREN1702131K>
- Liu, Y., Wang, E., Yang, X., & Wang, J. (2010): Contributions of climatic and crop varietal changes to crop production in the North China Plain, since 1980s. *Global Change Biology*, 16(8), 2287–2299. <https://doi.org/10.1111/j.1365-2486.2009.02077.x>
- Madić, M., Knežević, D., Đurović, D., Paunović, A., Stevović, V., Tomić, D., & Đekić, V. (2019): Assessment of the correlation between grain yield and its components in spring barley on an acidic soil. *Acta Agriculturae Serbica*, 24(47), 41-49. <https://doi.org/10.5937/AASer1947041M>
- Marković, S. M., Knežević, D., & Djukić, N. H. (2020): Effect of genotype and phenological stages on the accumulation rate of dry matter in wheat. *Journal of Animal & Plant Sciences*, 44(2), 7647-7656. <https://doi.org/10.35759/JAnmPlSci.v44-2.4>
- Matković, M., Bošković, J., Zečević, V., Knežević, D., & Đurić, N. (2015): Influence of genotype on yield and quality components of durum wheat in organic production. Fifth International Symposium on natural resources management, 23 May 2015, Zaječar, Serbia, Proceedings, 17-22.
- Matković Stojšin, M., Petrović, S., Banjac, B., Zečević, V., Roljević Nikolić, S., Đorđević, R., & Knežević, D. (2022a): Stability performances of different wheat genotypes grown under favorable and salinity stress conditions. Book of proceedings of the XIII International Scientific Agriculture Symposium “Agrosym 2022”, 6-9 October 2022, Jahorina, pp. 129-134.
- Petrović, S., Dimitrijević, M., Mihai, C.S., Bucur, D., Gurita, A.A., Harja, M., Maksimović, L., & Belić, M. (2012): Spike yield stability in wheat grown under stress conditions of alkaline soil. *Journal of Food Agriculture & Environment*, 10(2), 480-484. <https://doi.org/10.1234/4.2012.3033>
- Petrović, S., Dimitrijević, M., & Banjac, B. (2016): Variability and interrelationship of yield components in wheat grown on solonetz and chernozem Soil. *Letopis Naučnih Radova*, 40(1), 47-52.
- Saeidi, M., & Abdoli, M. (2015): Effect of drought stress during grain filling on yield and its components. gas exchange variables. and some physiological traits of wheat cultivars. *Journal of Agricultural Science and Technology*, 17, 885-898.
- Sreenivasulu, N., & Schnurbusch, T. (2012): A genetic playground for enhancing grain number in cereals. *Trends in Plant Science*, 17, 91–101. <https://doi.org/10.1016/j.tplants.2011.11.003>
- Sugár, E., Berzsenyi, Z., Árendás, T., & Bónis, P. (2016): Effect of nitrogen fertilization and genotype on the yield and yield components of winter wheat. *Die Bodenkultur: Journal of Land Management, Food and Environment*, 67(1), 25-34. <https://doi.org/10.1515/boku-2016-0003>
- Šumaruna, M., Mikić, S., Mladenov, V., Boćanski, J., Šućur, R., & Trkulja, D. (2022): Evaluation of and variability in yields and yield components of wheat cultivars in northern Serbia. *Contemporary Agriculture*, 71(1-2), 127-136. <https://doi.org/10.2478/contagri-2022-0018>
- Urošević, D., Knežević, D., Matković Stojšin, M., Laze, A., Branković, G., Stojiljković, J., & Zečević, V. (2025): Variability of mass of spike among wheat varieties. Book of proceedings of the XVI International Scientific Agriculture Symposium “Agrosym 2025”, 6-9 October 2025, Jahorina, pp. 277-284.
- Valerio, I. P., Carvalho, F. I. F., Oliveira, A. C., Benin, G., Souza, V. Q., Machado, A. A., Bertan, I., Busato, C. C., Silveira, G., & Fonseca, D. A. R. (2009): Seeding density in wheat genotypes as a function of tillering potential. *Scientia Agricola*, 66(1), 28-39. <https://doi.org/10.1590/S0103-90162009000100004>
- Würschum, T., Leiser, W.L., Langer, S.M., Tucker, M.R., & Longin, C. F. H. (2018): Phenotypic and genetic analysis of spike and kernel characteristics in wheat reveals long-term genetic trends of grain

- yield components. *Theoretical and Applied Genetics*, 131(10), 2071-2084. <https://doi.org/10.1007/s00122-018-3133-3>
- Xie, Q., Mayes, S., & Sparkes, L. D. (2016): Optimizing tiller production and survival for grain yield improvement in a bread wheat spelt mapping population. *Annals of Botany*, 117(1), 51–66. <https://doi.org/10.1093/aob/mcv147>
- Xie, Q., Li, N., Yang, Y., Lv, Y., Yao, H., Wei, R., et al. (2018): Pleiotropic effects of the wheat domestication gene *Q* on yield and grain morphology. *Planta*, 247, 1089–1098. <https://doi.org/10.1007/s00425-018-2847-4>
- Zečević, V., Knežević, D., Pavlović, M., & Mićanović, D. (1997): Genetic analysis of yield components in winter wheat (*Triticum aestivum ssp. vulgare* L.). *Genetika*, 29(1), 31-40.
- Zečević, V., Matković, M., Petrović, S., Dimitrijević, M., & Knežević, D. (2016): Stability of spike weight and grain weight per spike of different wheat genotypes. Book of proceedings of the VII International Scientific Agriculture Symposium, "Agrosym 2016", 6-9 October 2016, Jahorina, Bosnia and Herzegovina. pp. 587-593
- Zečević, V., Milenković, S., Bošković, J., Roljević Nikolić, S., Luković, K., Đorđević, R., & Knežević, D. (2021): Influence of foliar nutrition on yield and yield components of durum wheat (*Triticum durum* Desf.) grown in system of organic production. *Applied Ecology and Environmental Research*, 20(1), 171-187. http://dx.doi.org/10.15666/aeer/2001_171187

Frequency of the Fibonacci sequence of disc flowers in four genotypes of sunflower

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Abstract

The disc-flowers on the sunflower head are formed spirally in two directions. One clockwise and the other counterclockwise. In the experiment carried out in 2024 and 2025 at two locations in Hungary, we analysed the frequency of members of the Fibonacci sequence on four sunflower genotypes. We focused our research on two sequence categories: N + NB (N=exact number of the Fibonacci sequence and NB= N-1, N+1, N-2, N+2, N-3, N+3) and NoF – outside the N+NB category. Data for all eight groups were part of our research. The frequency of occurrence of Fibonacci sequence categories also depends on genotype, location, and year of testing. The experiment involved a large number of samples and required high-quality field trials. The research examined several characteristics of sunflower seeds to determine the relationship between the frequency of the Fibonacci sequence and these characteristics, and to explore their potential use in sunflower breeding. The experiment clearly showed the tendency of sunflower to form spirals closer to the Fibonacci sequence in the sunflower heads, but in much lower frequency than reported in the literature. We have concentrated on the Fibonacci sequence in our research, although the data can be used to determine the frequency of other sequences, such as the Lucas or F4 sequence.

Keywords: sunflower, Fibonacci sequence, clockwise, counterclockwise, location, year

INTRODUCTION

Leonardo Fibonacci was an Italian mathematician born in Pisa in the 13th century and is probably most responsible for introducing Arabic numerals in Europe. He is considered one of the most important mathematicians of the Middle Ages. In the Fibonacci sequence, each subsequent member of the sequence is equal to the sum of the previous two: 0, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, 144, etc... The Fibonacci sequence is related to the Golden Ratio because the limit of the ratio between predecessor and successor is approximately 0.618, while the limit of the ratio between successor and predecessor is approximately 1.618. There are many examples of the existence of the Fibonacci sequence in nature. The golden angle is a common example in phyllotaxy. Often, the arrangement of leaves on the stems of different plant species is related to the Fibonacci sequence, due to the angle between them of approximately 137.5°, the angle subtended by the smaller of the two arcs of a circle divided by the Golden Ratio. As stated by Okaba (2015), this angle is probably the optimal solution due to the least energy consumption. Schneider et al. (2014) observed that apple trees that have a good yield show a good and balanced Fibonacci sequence relationship for the angles of the leaves on the apple sapling. The disc flowers on the sunflower head are formed spirally in two directions: clockwise and counterclockwise. Alan Turing, a mathematical genius known to the public as a member of the group at Bletchley Park, in his later research devoted himself to the study of mathematical rules in nature, where he also proved the existence of the Fibonacci sequence in a sunflower head. Excluding this work done by Swinton et al., published in 2016, there are quite a few experiments in this field of research on sunflower and significantly earlier (Weisse, 1897; Schoute, 1938). The results of the "Turing Sunflower Project" based on citizens' experiments have shown a very high frequency of correct Fibonacci sequences of up to 74%.

The primary aim of this study was to examine the frequency of Fibonacci sequence patterns in sunflower disc flowers across different genotypes, locations, and years (2024–2025). A secondary objective was to explore potential correlations between these patterns and seed characteristics relevant for breeding. Understanding such relationships may contribute to the identification of useful traits and the development of improved sunflower genotypes.

MATERIALS AND METHODS

The experiment was set up at two locations in Hungary near the city of Pécs, in 2024 and 2025. In 2024, one location was near the village of Kozarmisleny (in the text KOZ) and the other near the village of Szentlőrinc (in the text SZL). While in 2025, locations were near Kozarmisleny and Boly (in the text BOY). Planting was carried out in the second half of April under optimal conditions. Four promising sunflower hybrids created in the breeding program of the KWS company were tested. In all locations, the production technology was identical, and randomization was conducted using a completely randomized design (CRD). The experiment was set up in three replicates. Approximately 70 plants per plot were marked with labels, from which the remnants of trumpet flowers were first cleaned at the R 6-7 stage of development (Schneider and Miller, 1981) and then photographed. At the KOZ location in 2025, photographs of the sunflower heads were taken at a later stage than optimal, leading to more data being discarded as unreliable, whereas the BOY location had extremely high-quality data. The total number of processed data in both years is 3904, which represents one of the largest samples reported in the literature. Each photograph of the head was printed and then analysed by counting the spirals in the clockwise direction, as well as in the counterclockwise direction (Fig. 1). We focused our research on two sequence categories: N + NB (N=exact number of the Fibonacci sequence and NB= N-1, N+1, N-2 and N+2, N-3 and N+3) and NO – outside the N+NB category. All eight groups were included in the analysis. In the year 2024, only 3.6% of the data were excluded from the data processing. In the year 2025, only 2.2% of the data were excluded from the location BOY and 80% from the KOZ location due to late photography. In the process of counting spirals, it is very important to take photos at the right moment, which was not the case in the KOZ location in 2025. In some cases, it was necessary to use a magnifying glass. Each photographed head of sunflower was picked by hand at full maturity, and in each head, seeds were individually separated to carry out further tests related to the number and quality of grains. Yield of the grain/ha was not the focus of this research. Every trial, including this one, must satisfy all qualitative criteria from the aspect of agronomic practice.

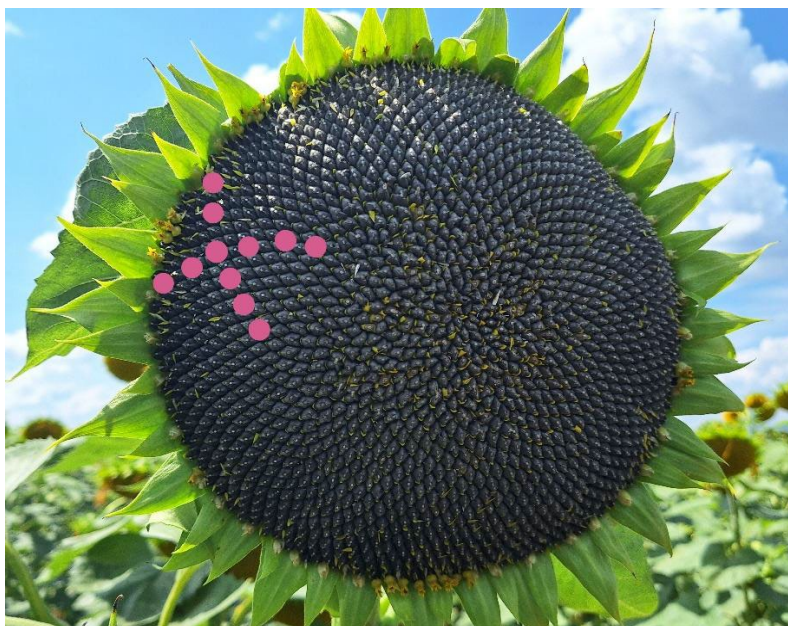


Fig. 1. Determination of the number of spirals in the clockwise direction, as well as in the counterclockwise direction, on a sunflower disk

RESULTS AND DISCUSSION

By comparing the frequency of the Fibonacci sequence during two years of testing on the same sunflower genotype, the difference between the exact Fibonacci sequence is clearly observed. It is significantly lower in 2024 than in 2025. Differences are observed, although they are not so pronounced in frequency in practically all categories between the number of clockwise and counterclockwise spirals (Fig 2a and 2b). The total frequency of the correct number of the Fibonacci sequence for both years of the study is only 24.8%, which are significantly lower values than the scarce reported in the literature for the number of spirals in the sunflower disc (Fig 3a). However, if we consider the whole group N+NB, which includes spiral numbers very close to the exact number of the Fibonacci sequence, it is quite clear that the sunflower tends to form spirals according to the Fibonacci sequence. In that case, the frequency of group N+NB is 62.5% (Fig. 3b).

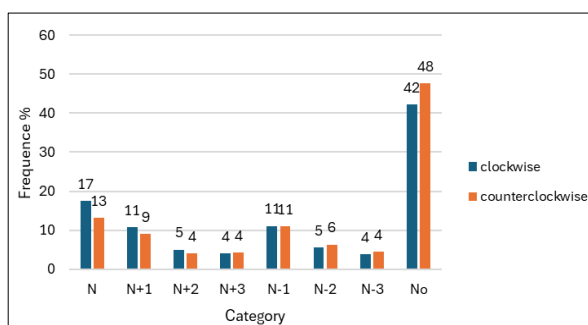


Figure 2a. Frequency of Fibonacci sequence categories in 2024

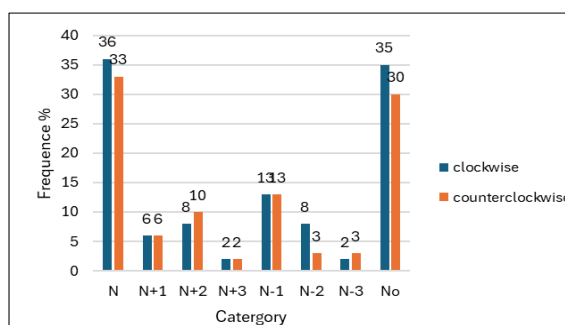


Figure 2b. Frequency of Fibonacci sequence categories in 2025

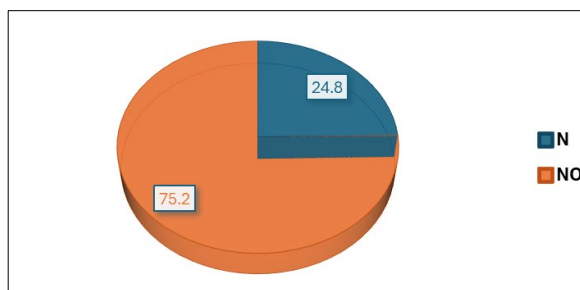


Figure 3a. Average frequency (%) of N and NO group in both tested years

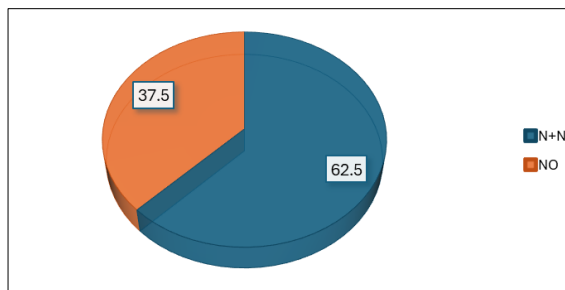


Figure 3b. Average frequency (%) of N+NB group in tested years

The results indicate at the frequencies of the three observed groups (N, N+NB and NO), the results show that the three hybrids have similar values, while the hybrid KHC215121 has significantly higher values, which shows us that the frequency of the Fibonacci sequence also depends on the genotype on which we are testing (Fig 4). The results of the experiment also showed that there are differences in frequency between the categories of the Fibonacci sequence and between the examined years (Fig. 5), although we must consider that the locations in the two years were not identical, but geographically they were very close to each other. In this type of experiment, it is important to note that locations are never identical because we must consider crop rotation. If we were to observe the frequency of occurrence of the Fibonacci sequence in both years, the number 89 dominates, followed by the number 55, the numbers 34 or 144 appear in extremely low frequency.

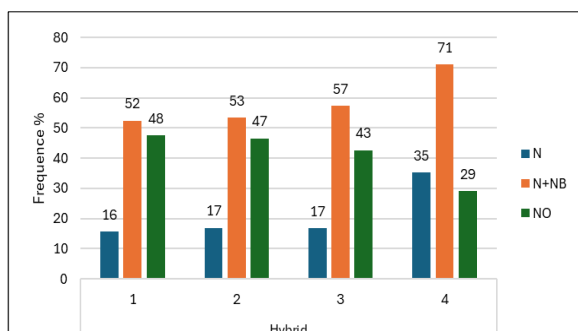


Figure 4. Average frequency of three groups by hybrid in the two tested years

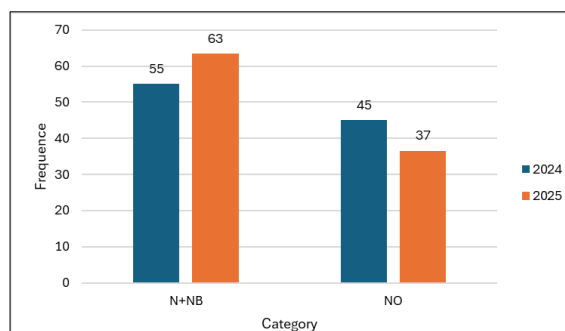


Figure 5. Frequency (%) of two groups by years

Of course, it is possible to analyse the results on the frequency of occurrence of the exact numbers of the Fibonacci sequence 55 and 89, both by locations and by years and genotypes, but we did not analyse that in this paper. ANOVA for both years are presented in Table 1a. and 1b. First, we transform the frequency results that are in percentages to improve the normality of the distribution, preventing the effect of borders (0% and 100%). The transformation was done according to the formula: $=\text{ASIN}(\text{SQRT}(x/100))$. The Year factor had a significant influence in the N+NB group, regardless of whether it is the frequency of clockwise or counterclockwise spirals. The F-critical is derived from the model and the F-statistic from the data.

Table 1a. Variance analysis (ANOVA) of the frequency of the Fibonacci sequence for the N+NB group for two years of testing and clockwise

Source of Variation	SS	df	MS	F	P-value	F crit
Year	0.33	1.00	0.33	7.21	0.02	4.49
Genotype	0.20	3.00	0.07	1.44	0.27	3.24
Interaction	0.14	3.00	0.05	1.03	0.40	3.24
Within	0.73	16.00	0.05			
Total	1.40	23.00				

Table 1 b. Variance analysis (ANOVA) of the frequency of the Fibonacci sequence for the N+NB group for two years of testing and counterclockwise

Source of Variation	SS	df	MS	F	P-value	F crit
Year	0.29	1.00	0.29	7.56	0.01	4.49
Genotype	0.22	3.00	0.07	1.94	0.16	3.24
Interaction	0.13	3.00	0.04	1.13	0.36	3.24
Within	0.62	16.00	0.04			
Total	1.26	23.00				

Empirical research on the occurrence of Fibonacci in sunflowers has so far been limited to a small number of research papers, such as those by Shoute (1938), Majumder and Chakravarti (1976), or Swinton et al. (2016). So far, the most extensive work has been by Swinton et al., based on citizen research. They divided the project into four phases: plant, cultivate, measure, and count. However, the authors themselves noted that the main problem with such a citizen experiment is the lack of homogeneity, which led to a very different distribution of samples across several members of the Fibonacci sequence: 21, 34, 55, and 89. Even the authors themselves stated that the biggest problem with the citizen experiment is the large number of samples from different sources, and it is easier to measure variability than to find regularities. A larger number of samples in such a citizen's experiment were not usable due to damage or disease of the heads, because although there were excellent instructions, the training of citizens to perform such an experiment was very varied. The process of plant breeding actively uses the results of evolution but then directs them further to meet human needs. Often,

these needs are different from evolutionary movements that are more directed towards better adaptation and the spread of the species. Although we used four genotypes that differ according to our internal standards, their genetic variability remains narrow. This may have influenced the appearance of a significantly lower frequency of correct members of the Fibonacci sequence than in other studies, especially in Swinton et al. (2016). Nevertheless, the experiment we performed in 2024 and 2025 clearly showed the presence of the Fibonacci sequence in sunflower heads. The spiral way of packing seeds in the head is probably the most efficient way, but the question arises whether there are evolutionary advantages for those genotypes that have a higher frequency of the Fibonacci sequence, and what is that advantage? The results of the research by Dozet and Kristof (2025) showed that there was no significant correlation between the examined seed characteristics and the frequency of the Fibonacci sequence, including the number of seeds per head. The number of grains per head and the weight of 1000 grains are strongly correlated with seed yield, so it is quite possible that selection for yield in hybrids actually favored more lines (and thus the hybrid) with a lower frequency of the Fibonacci sequence. It is very possible that the selection of lines led not only to the narrowing of genetic variability but also to a necessary and negligible effect on the frequency of the Fibonacci sequence in the tested genotypes. As we showed in the two-year experiment, there are significant differences between genotypes in the frequencies of the Fibonacci sequence; the question of the genes that control this property also arises. Although there are numerous studies in sunflower on the identification of genes that participate in the expression of many properties, unfortunately, we did not find a single work dedicated to the identification of genes that control the appearance of the Fibonacci sequence. Such works are rare even in the phyllotaxy of other plant species, but they clearly indicate the role of genetic control of the formation of spirals close to the golden angle (137.5°). Results of Takashi et al. (2002) show that disruption of the *Arabidopsis* topoisomerase (topo) I gene, *TOP1 α* , affects phyllotaxy and plant architecture. Perhaps such research has not been done on sunflower, but it has been done on other related species, such as gerberas. Zhang et al. (2021) integrated experimental data and computational models to explain phyllotaxis in *Gerbera hybrida*. Results showed that phyllotactic patterning in *Gerbera* is regulated by changes in the size of the morphogenetically active zone coordinated with head growth. Their results proved the essential role of growth dynamics and the active zone in flower head patterning. According to Elomaa (2021), during plant growth, the main role is played by auxin, whose levels reach maximum simultaneously in several locations of the meristem, which is the main reason Fibonacci spirals are formed. Research on *Arabidopsis* or *Gerbera* is the real guide to where research should go in sunflowers, but there are still many pieces of the puzzle that need to be put in place, especially at the molecular level. As it has been shown many times in various experiments, auxin biosynthesis is influenced by external factors such as drought, high air temperatures, lighting, or the content of nutrients in the soil. Several authors have proven in experiments that auxins play a key role in organogenesis, and thus in the formation of spirals that follow the Fibonacci sequence. When we know all that, then it is probably very explainable that the frequency of the Fibonacci sequence in the experiment we performed during 2024 and 2025 varies depending on the year, locality, and genotype.

CONCLUSION

Inspired by the work of the great mathematician Alan Turing, who demonstrated the existence of the Fibonacci sequence in the arrangement of flowers in a sunflower head, and then by the work of Swinton et al. (2016) in a very specific citizen experiment, we conducted an experiment on four sunflower genotypes, in two years of testing and in two localities. We wanted to determine whether there are differences between genotypes, the influence of year and locality on the frequency of the Fibonacci sequence. This very demanding experiment that generated more than 2,000 photos taken directly from the field and generated more than 4,000 data points, where we determined their frequency by counting spirals in a clockwise direction as well as counterclockwise direction, showed us that this frequency depends on the genotype itself, the year, and the locality. The goal of our broader research was not only oriented to the frequency of the Fibonacci sequence that we presented in this paper, but also to determine the correlation with some seed characteristics, such as the number of seeds per head, the weight of one thousand seeds, and even oil content. We will publish those results in the future. When looking at the frequency of the exact members of the Fibonacci sequence, number 89 appeared with the highest frequency, followed by number 55. Exact matches to the numbers in the Fibonacci sequence were

relatively rare in our experiment. If we compare our results with the results of Swinton et al. (2016), which show much higher divergence, they worked with much more divergent material than we did in this paper. The frequency of the exact number of the Fibonacci sequence is still higher for clockwise spirals compared to counterclockwise. There are deviations from the exact Fibonacci sequence, and in our work that the deviations that we classified in the NB (nearby) category also showed us that it, together with the frequency of the exact number (N) of the Fibonacci sequence, is high (62.5%), which clearly confirms earlier research that sunflowers form flowers according to the Fibonacci sequence. Considering the results we published in 2024, for those of us who are involved in sunflower breeding, it remains a mystery whether the formation of flowers and seeds favours genotypes that have a higher frequency of categories N and NB, or maybe vice versa. It is true that evolution is not always in line with breeding goals. We hope that our research on sunflower will inspire other researchers to devote themselves to this topic and to find answers to the many open questions of phenomena for the phyllotaxy of flowers on sunflower heads.

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REFERENCES

- Dozet, B., & Poor, K. (2025). Relation of the Fibonacci sequence with some characteristics of sunflower seeds. *Proceedings of 66th Oil Industry Conference Production and Processing of Oil Seeds*, 32–42.
- Eloma, P. (2021). Auxin makes the spirals in gerbera inflorescences follow the Fibonacci sequence. *PhysOrg*, April 14, 2021.
- Majumder, P. P., & Charavarti, A. (1976). Variation in the number of ray- and disc-florets in four species of *Composita*. *The Fibonacci Quarterly*, 14(2), 97–10. <https://doi.org/10.1080/00150517.1976.12430560>
- Okabe, T. (2016). Biophysical optimality of the golden angle in phyllotaxis. *Scientific Reports*, 5, 15358. <https://doi.org/10.1038/srep15358>
- Schoute, J. C. (1938). On whorled phyllotaxis. IV. Early binding whorls. *Recueil des Travaux Botaniques Néerlandais*, 35(1), 415–558.
- Šnajder, I., Čmelik, Z., Radišić, B., Zima, D., Zelić, J., & Šnajder J. (2014). Correlation between the Fibonacci sequence to a one-year old seedling and yield load of apples. 49th Croatian & 9th International Symposium on Agriculture, Dubrovnik, Croatia, *Proceedings*, 718–721.
- Swinton, J., Ochu, E., The MSI Turing's Sunflower Consortium (2016). Novel Fibonacci and non-Fibonacci structure in the sunflower: results of a citizen science experiment. *Royal Society Open Science*, 3 (5): 160091. <https://doi.org/10.1098/rsos.160091>
- Takahashi, T., Matsuhara, S., Mitsutomo, A., & Komeda, Y. (2002). Disruption of a DNA topoisomerase I gene affects morphogenesis in Arabidopsis. *Plant Cell*, 14(9), 2085–2093. <https://doi.org/10.1105/tpc.001925>
- Weisse, A. (1897). Die Zahl der Randblüthen an Compositenköpfchen in ihrer Beziehung zur Blattstellung und Ernährung. *J. Wiss. Botanik*, 30, 453–483.
- Zhang, T., Cieslak, M., Owens, A., Wang, F., Broholm, S. K., Teeri, T. H., Elomaa, P., & Prusinkiewicz, P. (2021). Phyllotactic patterning of gerbera flower heads. *Proceedings of the National Academy of Sciences of the United States of America*, 118(13), e2016304118, <https://doi.org/10.1073/pnas.2016304118>

Organic mulches as a conservation agriculture practice in organic turmeric (*Curcuma longa* L.) production

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Abstract

The introduction of turmeric (*Curcuma longa* L.) into temperate-continental environments requires the development of sustainable soil management practices adapted to organic production systems. This pilot study evaluated the effects of three soil management treatments - bare soil, straw mulch, and sheep wool mulch - on growth parameters, soil conditions, and rhizome yield of turmeric cultivated in an organic greenhouse at the Biotechnical Centre Naklo (Slovenia) during the 2025 growing season. Organic mulching significantly improved plant growth compared to bare soil. Plants grown under straw and sheep wool mulch exhibited greater height, larger leaf area, and thicker stems. Sheep wool provided the most stable soil moisture conditions (31.5%) and the lowest weed infestation (16.1 plants m⁻²), resulting in the highest total fresh rhizome yield (7.7 kg m⁻²) and dry matter content (26.8%). Bare soil treatments showed significantly lower soil moisture, higher weed pressure, and the lowest yield (4.4 kg m⁻²). The results confirm that organic mulches are an effective conservation agriculture practice for turmeric production under temperate greenhouse conditions. Sheep wool, as a locally available and biodegradable material with high water retention capacity, proved particularly suitable for enhancing soil stability, reducing weed pressure, and increasing yield. These findings provide a basis for developing sustainable production technologies for high-value specialty crops in non-tropical environments and support the diversification of organic greenhouse systems.

Keywords: *Curcuma longa*, organic mulching, weed suppression, rhizome yield, conservation agriculture

INTRODUCTION

In recent decades, European agriculture has been facing increasing challenges, most notably climate change, restrictions on the use of synthetic plant protection products, and growing demands for sustainable and environmentally friendly production systems (Verma & Pradhan, 2024). These factors stimulate the search for alternative high-value crops that can enhance the resilience of agroecosystems while also providing economic and nutritional potential. One such crop is turmeric (*Curcuma longa* L.), a tropical species from the Zingiberaceae family, known for its high content of curcuminoids with pronounced antioxidant and anti-inflammatory properties (Hewlings & Kalman, 2017).

Traditional turmeric cultivation is largely limited to tropical and subtropical regions; however, increasing global demand is encouraging research into its production under temperate-continental climatic conditions (Aydişakir et al., 2025). When introducing this crop into new agroecological environments, it is essential to understand the effects of cultivation practices, particularly those based on the principles of conservation agriculture, such as soil protection, reduction of degradation processes, and promotion of soil biological activity.

The use of organic mulches represents one of the fundamental measures of conservation soil management. Mulching reduces water evaporation, stabilizes soil temperature, suppresses weed growth,

and enhances biological activity - factors that are particularly important in protected cultivation systems. In tropical regions, studies have already shown that various organic materials, such as straw or rice straw, positively affect growth parameters and turmeric yield (Iqbal et al., 2020).

Research on the effects of mulching on soil physical and biological properties confirms that different organic materials can increase moisture retention, reduce water losses, and stimulate soil organism activity (Kader et al., 2017; Akchaya et al., 2025). These effects are particularly relevant in organic greenhouses, where the use of chemical inputs is limited, making mulching a key practice for long-term soil fertility and stability (Verma & Pradhan, 2024).

Despite extensive literature on the impact of mulching on various agricultural crops, data on the response of turmeric to specific types of organic mulches under temperate-continental conditions remain scarce. The knowledge gap is even more pronounced in protected cultivation systems, where higher temperatures, accelerated mineralization of organic matter, and altered soil moisture dynamics may lead to plant responses that differ from those observed in tropical environments (Aydınşakir et al., 2025). Understanding these responses is crucial for the development of efficient and sustainable cultivation technologies that enable stable and high-quality turmeric production in new environments.

The aim of this pilot study was to evaluate the effects of three soil management technologies - bare soil, straw mulch, and sheep wool mulch - on the growth parameters and yield of turmeric in an organic greenhouse at the Biotechnical Centre Naklo (Slovenia). Sheep wool, as a mulching material, represents a locally available, biodegradable, and sustainable alternative which, due to its structure and moisture retention capacity, may significantly contribute to conservation soil management in greenhouse systems. The working hypothesis was that the application of organic mulches improves turmeric growth and yield through a more favorable water regime, reduced weed pressure, and more stable soil conditions, with effects differing depending on the material used.

The results of this study will contribute to a better understanding of the potential for turmeric cultivation in temperate-continental climates and provide an important basis for the development of sustainable production technologies for high-value specialty crops. Furthermore, they will support efforts to enhance self-sufficiency, diversify production systems, and promote the efficient use of locally available organic materials in organic and conservation agriculture.

MATERIAL AND METHODS

The experiment was conducted from 10 May to 20 November 2025 in an organic greenhouse at the Biotechnical Centre Naklo (420 m a.s.l.; 46°17'12" N; 14°18'36" E). The greenhouse floor was divided into nine plots, each measuring 1.5 m × 2.0 m (3.0 m²). Three soil management treatments - bare soil, straw mulch, and sheep wool mulch - were assigned to plots in a randomized complete block design with three replications.

Before transplanting, 0.8 kg m⁻² of the organic-mineral fertilizer Tiger DUNG (3–6–12 + 2 MgO + 14 SO₃) was incorporated into the top 15 cm of soil using a rotary tiller. The soil surface was leveled manually to ensure uniform planting conditions.

Turmeric seedlings (Madras type) were transplanted at a spacing of 30 cm × 50 cm.

Irrigation was supplied through drip lines equipped with pressure-compensated emitters spaced at 30 cm (2 L h⁻¹). Irrigation was applied twice weekly during establishment and adjusted to 1-3 weekly applications thereafter, depending on greenhouse temperatures and soil moisture status. Each irrigation event delivered 5-8 L m⁻² uniformly across all treatments. No supplemental fertigation was used.

Mulches were applied immediately after transplanting: straw mulch (wheat straw, applied at a thickness of 6-7 cm, equivalent to approx. 2.5 kg m⁻², fresh weight) and sheep wool mulch (locally sourced raw wool, unwashed but shaken free of large contaminants, applied at 1.2 kg m⁻² and spread to a thickness of 3-4 cm). Mulches were not replenished during the season.

Vegetative growth measurements were taken 150 days after transplanting, corresponding to peak vegetative development, including plant height (from soil surface to the tip of the highest fully expanded leaf), number of fully developed leaves, leaf area (estimated using a LI-3000C meter on three upper

canopy leaves and scaled to whole-plant leaf number), and stem diameter measured 5 cm above the soil surface; all measurements were conducted on five randomly selected plants per plot.

Soil moisture was monitored weekly using an SM150 dielectric probe inserted at 10 cm depth, with three readings per plot averaged. Weed infestation was assessed at midseason (90 days after transplanting) using a 0.25 m² quadrat placed at three randomly selected non-overlapping positions per plot, counting all emerged weeds and converting values to plants m⁻².

Rhizomes were harvested manually at physiological maturity (20 November 2025), washed, weighed fresh, and plot yields converted to kg m⁻². For dry matter determination, a 200 g rhizome subsample per plot was oven-dried at 65 °C for 72 hours to constant weight, and dry matter (%) calculated as (dry mass/fresh mass) × 100.

Data were analyzed using IBM SPSS Statistics v.29 with one-way ANOVA, and significant differences among treatments were separated using Tukey's HSD test at $P < 0.05$.

RESULTS AND DISCUSSION

Vegetative growth

Clear differences in vegetative development of turmeric were observed among the three soil management treatments (Table 1). Although plants grown under straw and sheep wool mulch were numerically taller (158.4 cm and 162.3 cm, respectively) than those on bare soil (141.6 cm), these differences were not statistically significant. A similar pattern was observed for the number of leaves, where mulched plants showed slightly higher values, but the differences did not reach statistical significance. In contrast, leaf area and stem diameter were both significantly increased by mulching ($P < 0.05$).

Leaf area and stem diameter exhibited similar treatment-dependent trends. The smallest average leaf area was measured in bare soil plants (320.8 cm²), whereas straw mulch increased leaf area to 450.2 cm², and sheep wool mulch resulted in 438.6 cm². Stem diameter followed the same pattern: 10.2 mm in the bare soil treatment compared with 14.0 mm under straw mulch and 13.8 mm under sheep wool. According to the statistical analysis, both mulching treatments significantly increased leaf area and stem diameter relative to bare soil ($P < 0.05$).

The enhanced morphological development under mulching suggests that mulch layers created more favorable microclimatic conditions in the root zone. Higher soil moisture availability and reduced surface temperature fluctuations likely contributed to enhanced leaf expansion and stem thickening. These effects align with known physiological responses of turmeric and other rhizomatous crops, which depend on stable soil moisture and moderated temperature regimes to sustain vegetative growth. The slightly greater performance under sheep wool mulch indicates that its higher moisture-retention capacity provided the most favorable conditions for achieving maximal vegetative biomass.

Table 1. Growth parameters of turmeric in different treatments

Treatment	Plant height (cm)	No. of leaves	Leaf area (cm ²)	Stem diameter (mm)
Bare soil	141.6	10.6	320.8 a	10.2 a
Straw	158.4	11.2	450.2 b	14.0 b
Sheep's wool	162.3	11.4	438.6 b	13.8 b

Values followed by different letters are significantly different at $P < 0.05$. The absence of letters indicates that there is no statistical difference between the treatments.

Soil moisture and weed suppression

The primary agronomic benefit of mulching in this study was the stabilization of the root-zone microenvironment. Soil moisture was markedly higher under sheep wool (31.5%) and straw (23.4%) compared with bare soil (15.6%), demonstrating a clear reduction in evaporative water loss. This outcome aligns with established evidence that mulches limit soil - atmosphere energy exchange by intercepting solar radiation and moderating soil temperature fluctuations (Teasdale & Mohler, 2000; Li et al., 2013). Reduced thermal and radiative fluxes weaken vapor pressure gradients at the soil surface, thereby restricting capillary rise and surface evaporation and preserving water within the rhizosphere. In temperate-continental greenhouse environments, where diurnal temperature oscillations can be substantial, such buffering is essential for maintaining root-zone stability (Steinmetz et al., 2016). Sheep wool exerted the strongest effect due to its fibrous, porous structure and high hygroscopicity. Wool fibers can absorb and retain moisture from both soil and air, releasing it gradually under drier conditions. Acting as a dynamic moisture reservoir, wool mulch mitigates short-term water deficits and helps maintain root hydraulic conductivity, supporting efficient water uptake and sustained transpiration (Comas et al., 2013). Stable moisture conditions are particularly important for rhizomatous crops such as turmeric, where continuous assimilate transport to underground storage organs depends on consistent plant water status.

Mulching also reduced weed density by more than 80% relative to bare soil. This suppression results primarily from restricted light penetration, which limits photosynthetically active radiation available for weed seed germination and early seedling growth (Teasdale & Mohler, 2000). Altered soil temperature and moisture regimes further influence weed emergence patterns and seedbank dynamics (Chauhan & Johnson, 2010). Beyond physical suppression, reduced weed pressure has important physiological implications for the crop. Without competition for water, nutrients, and light, turmeric can allocate more assimilates to vegetative growth and rhizome development. Early-season competition for nitrogen and soil moisture is known to reduce crop growth rate, radiation interception, and biomass accumulation (Rajcan & Swanton, 2001). Under bare soil conditions, the high weed density (95.6 plants m⁻²) likely intensified belowground competition, limiting nutrient diffusion and increasing rhizosphere overlap between crop and weeds (Casper & Jackson, 1997). Such resource limitation induces stress responses that alter carbon allocation and increase metabolic costs (Poorter et al., 2012). Consequently, yield differences among treatments reflect not only improved soil moisture retention under mulch but also reduced interspecific competition, which enhances carbon assimilation efficiency and promotes storage organ development.

Table 2. Soil moisture and weed infestation

Treatment	Average soil moisture (%)	Weed infestation (no. / m ²)
Bare soil	15.6 a	95.6 b
Straw	23.4 b	28.4 a
Sheep's wool	31.5 c	16.1 a

Values followed by different letters are significantly different at P<0.05. The absence of letters indicates that there is no statistical difference between the treatments.

Rhizome yield and quality

The highest fresh rhizome yield was obtained under sheep wool mulch, followed by straw, whereas the lowest yields were recorded in the bare soil treatment. The differences among treatments were statistically significant (P < 0.05), indicating that soil surface management substantially influenced productivity. In addition to higher total yield, both mulching treatments increased the proportion of marketable rhizomes, suggesting improved uniformity of underground organ development and reduced morphological variability within plots. Uniform rhizome formation is typically associated with stable soil moisture and temperature conditions during tuberization and bulking phases (Kader et al., 2017).

The yield enhancement observed under mulching can be attributed to multiple interacting mechanisms. First, improved soil water availability under mulch reduces plant water stress and maintains stomatal conductance, thereby sustaining photosynthetic carbon assimilation (Iqbal et al., 2020). Continuous assimilate supply is particularly critical for rhizomatous crops, where storage organ development depends on sustained carbohydrate translocation from source leaves to sink tissues. Second, substantial weed suppression under mulched treatments likely minimized belowground competition for nitrogen and water. Reduced interspecific competition enhances nutrient uptake efficiency and increases the fraction of assimilates allocated to economically important organs rather than stress-response mechanisms (Rajcan & Swanton, 2000). Third, mulch moderates soil temperature fluctuations, preventing extreme thermal stress that can disrupt enzymatic processes involved in carbohydrate metabolism and sink strength regulation (Steinmetz et al., 2016). Stable soil temperature conditions promote consistent rhizome enlargement and reduce physiological disorders associated with thermal variability. Fourth, organic mulches stimulate soil microbial activity by supplying carbon substrates, thereby enhancing nutrient mineralization and availability (Bonanomi et al., 2014). Increased microbial activity improves nitrogen cycling and may enhance root growth and nutrient acquisition capacity.

In the case of sheep wool, additional agronomic benefits may derive from its biochemical composition. Wool consists primarily of keratin, a nitrogen-rich structural protein. During gradual microbial decomposition, keratin can release ammonium and other nitrogenous compounds, functioning as a slow-release nitrogen source (Hadas et al., 2008; Nustorova et al., 2005). Such sustained nitrogen availability during later growth stages may have stimulated rhizome bulking and increased dry matter accumulation. Similar responses were reported in turmeric cultivated under non-tropical conditions, where improved nutrient supply and soil moisture stabilization significantly enhanced rhizome yield (Aydişakir et al., 2025).

Collectively, these findings indicate that yield improvements under mulching cannot be attributed to a single factor but rather to synergistic interactions among hydrological regulation, thermal buffering, nutrient cycling, and reduced competitive stress. Sheep wool, due to its combined hygroscopic, insulating, and nitrogen-releasing properties, appears to integrate these mechanisms more effectively than straw, thereby maximizing yield and marketable rhizome proportion.

Table 3. Rhizome yield and quality

Treatment	Total fresh yield (kg/m ²)	Weight of marketable rhizomes (g/plant)	Dry matter (%)
Bare soil	4.4 a	524.4 a	20.4 a
Straw	7.4 b	885.6 b	23.5 ab
Sheep's wool	7.7 b	924.5 b	26.8 b

Values followed by different letters are significantly different at $P < 0.05$. The absence of letters indicates that there is no statistical difference between the treatments.

CONCLUSIONS

The results confirm that the use of organic mulch is an effective measure for improving turmeric production in temperate continental conditions. Although turmeric is a thermophilic plant, it responded well in the greenhouse to the stable soil conditions provided by straw and sheep's wool. The positive effects of sheep's wool were particularly pronounced, proving to be a very suitable material for organic greenhouses as it is locally available, biodegradable, and has a high moisture retention capacity.

Compared to tropical studies, where plant mulch was most commonly used, the results suggest that in temperate continental conditions, materials with greater insulating capacity (e.g., wool) may provide additional benefits. This is particularly important during periods of temperature fluctuations, which are more frequent in our conditions than in tropical environments.

Further research could focus on optimizing the thickness of mulch, as sheep's wool showed the highest soil moisture values in the experiment. while it would also be useful to examine wool decomposition and possible nitrogen release in more detail and compare its effectiveness with other locally available

materials. It would also be important to investigate the effect of mulch on soil microbial activity, to examine the long-term effects over several growing seasons, and to extend the experiments to different types of greenhouses and outdoor cultivation, where additional differences in plant response may become apparent. Further work could include analyses of rhizome quality, such as curcumin and essential oil content, and an economic assessment of the use of wool in practice, while a comparison of different turmeric varieties and interactions between mulches and irrigation systems would provide a more comprehensive understanding of their impact on growth and yield.

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REFERENCES

- Akchaya, K., Parasuraman, P., Pandian, K., Vijayakumar, S., Thirukumaran, K., Mustaffa, M. R. A. F., Rajpoot, S. K., & Choudhary, A. K. (2025). Boosting resource use efficiency, soil fertility, food security, ecosystem services, and climate resilience with legume intercropping: A review. *Frontiers in Sustainable Food Systems*, 9, 1527256. <https://doi.org/10.3389/fsufs.2025.1527256>
- Aydınşakir, K., Jayaweera, D. M., & Pradhan, S. (2025). Adaptation of turmeric (*Curcuma longa* L.) to non-tropical greenhouse conditions: Growth performance and nutrient dynamics. *Agricultural Water Management*, 298, 108925. <https://doi.org/10.17557/tjfc.1561441>
- Bonanomi, G., De Filippis, F., Cesarano, G., La Stora, A., Ercolini, D., & Scala, F. (2014). Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. *Soil Biology and Biochemistry*, 75, 327-336. <https://doi.org/10.1016/j.soilbio.2014.04.005>
- Casper, B. B., & Jackson, R. B. (1997). Plant competition underground. *Annual Review of Ecology and Systematics*, 28, 545-570. <https://doi.org/10.1146/annurev.ecolsys.28.1.545>
- Chauhan, B. S., & Johnson, D. E. (2010). The role of seed ecology in improving weed management strategies in the tropics. *Advances in Agronomy*, 105, 221-262. [https://doi.org/10.1016/S0065-2113\(10\)05006-6](https://doi.org/10.1016/S0065-2113(10)05006-6)
- Comas, L. H., Becker, S. R., Cruz, V. M. V., Byrne, P. F., & Dierig, D. A. (2013). Root traits contributing to plant productivity under drought. *Frontiers in Plant Science*, 4, 442. <https://doi.org/10.3389/fpls.2013.00442>
- Hadas, A., Kautsky, L., Goek, M., & Kara, E. E. (2008). Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. *Soil Biology and Biochemistry*, 40(9), 2375-2382. <https://doi.org/10.1016/j.soilbio.2003.09.012>
- Hewlings, S. J., & Kalman, D. S. (2017). Curcumin: A review of its effects on human health. *Foods*, 6(10), 92. <https://doi.org/10.3390/foods6100092>
- Iqbal, R., Raza, M. A. S., Valipour, M., Saleem, M. F., Zaheer, M. S., Ahmad, S., Toleikiene, M., Haider, I., Aslam, M. U., & Nazar, M. A. (2020). Potential agricultural and environmental benefits of mulches - A review. *Bulletin of the National Research Centre*, 44, 75. <https://doi.org/10.1186/s42269-020-00290-3>
- Kader, M. A., Senge, M., Mojid, M. A., & Ito, K. (2017). Recent advances in mulching materials and methods for modifying the soil environment. *Soil & Tillage Research*, 168, 155-166. <https://doi.org/10.1016/j.still.2017.01.001>
- Li, R., Hou, X., Jia, Z., Han, Q., Yang, B., & Huang, Y. (2013). Effects of plastic film mulch on soil water and temperature dynamics and maize yield in a semiarid region of northwest China. *Soil & Tillage Research*, 128, 78-86. <https://doi.org/10.1016/j.still.2012.10.012>

- Nustorova, M., Braikova, D., Gousterova, A., Vasileva-Tonkova, E., & Nedkov, P. (2005). Chemical, microbiological and plant analysis of soil fertilized with alkaline hydrolysate of sheep wool waste. *World Journal of Microbiology and Biotechnology*, 21, 77-83. <https://doi.org/10.1007/s11274-004-1556-0>
- Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist*, 193(1), 30-50. <https://doi.org/10.1111/j.1469-8137.2011.03952.x>
- Rajcan, I., & Swanton, C. J. (2001). Understanding maize–weed competition: Resource competition, light quality and the whole plant. *Field Crops Research*, 71(2), 139-150. [https://doi.org/10.1016/S0378-4290\(01\)00159-9](https://doi.org/10.1016/S0378-4290(01)00159-9)
- Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., Tröger, J., Muñoz, K., Frör, O., & Schaumann, G. E. (2016). Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? *Science of the Total Environment*, 550, 690-705. <https://doi.org/10.1016/j.scitotenv.2016.01.153>
- Teasdale, J. R., & Mohler, C. L. (2000). The quantitative relationship between weed emergence and the physical properties of mulches. *Weed Science*, 48(3), 385-392. [https://doi.org/10.1614/0043-1745\(2000\)048\[0385:TQRBWE\]2.0.CO;2](https://doi.org/10.1614/0043-1745(2000)048[0385:TQRBWE]2.0.CO;2)
- Verma, R., & Pradhan, S. (2024). Effect of Mulches on Crop, Soil and Water Productivity: A Review. *Agricultural Reviews*, 45(2), 335-339. <http://doi.org/10.18805/ag.R-2243>

Optimization of irrigation for improving seedling production of *Marrubium vulgare* L.

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Abstract

Marrubium vulgare L. is a perennial medicinal plant belonging to the Lamiaceae family, widely used in the pharmaceutical and herbal industries due to its expectorant, anti-inflammatory, and antioxidant properties. The production of high-quality seedlings represents a key prerequisite for successful cultivation and establishment of this species. The aim of this study was to evaluate the effect of different irrigation regimes on seedling emergence, stand formation, and morphological characteristics of *M. vulgare* L. seedlings under open-field nursery conditions. The experiment was conducted in 2025 at the Institute for Medicinal Plant Research “Dr Josif Pančić” in Pančevo, Serbia. Seeds were sown on 28 July at a sowing rate of 10 g m⁻², and transplanting was performed on 30 November. Three irrigation treatments were applied: no irrigation (T₀), moderate irrigation (T₁: 6 l m⁻² day⁻¹), and intensive irrigation (T₂: 10 l m⁻² day⁻¹). The results showed that irrigation significantly affected emergence and seedling morphology. Intensive irrigation provided the highest emergence (99.0%), but resulted in reduced seedling quality due to high plant density and competition. In contrast, the non-irrigated treatment produced the largest individual seedlings but had insufficient emergence (34.0%). Moderate irrigation ensured optimal seedling emergence (62.0%) and the best overall seedling quality, characterized by balanced shoot and root development. Based on the obtained results, moderate irrigation can be recommended as the optimal regime for the production of high-quality *M. vulgare* L. seedlings.

Keywords: white horehound, seedlings, irrigation, emergence, medicinal plants

INTRODUCTION

M. vulgare L. (white horehound) is a perennial medicinal plant belonging to the family Lamiaceae, originating from the Mediterranean region and Central Asia, and is currently widely distributed across almost all continents (Acimović et al., 2020). Owing to its pronounced pharmacological properties and the presence of bioactive compounds, particularly labdane-type diterpenes such as marrubiin, phenolic acids, and flavonoids, *M. vulgare* has significant importance in traditional and modern phytotherapy (Shakeri et al., 2016; Acimović et al., 2020). The most valuable plant part is the herb, i.e., the aboveground biomass, which is widely used for the production of herbal teas, extracts, and phytopreparations exhibiting expectorant, antiinflammatory, antispasmodic, and antioxidant activities (Basar et al., 2013).

The increasing demand for standardized and high-quality herbal raw material has led to a gradual transition from wild harvesting to the cultivated production of this species (Gurčik et al., 2005). Under the agroecological conditions of Serbia, *M. vulgare* has proven to be a highly adaptable and relatively undemanding crop, suitable for cultivation within diverse production systems (Kišgeci, 2008). However,

the success of later cultivation stages, including yield and herb quality, largely depends on the quality of planting material, making seedling production a critical component of the overall cultivation technology of medicinal plants (Letchamo et al., 2006).

Seedling production of medicinal plants is most commonly conducted under controlled or semi-controlled conditions, such as greenhouses, glasshouses, and open cold frames (Cantliffe et al., 2007). Open cold frames represent a low-input and economically viable nursery system, particularly important from the perspective of reduced energy consumption and gradual acclimatization of seedlings to outdoor environmental conditions (Raviv and Lieth, 2008). From a socio-economic standpoint, seedling production in open cold frames represents a sustainable solution for small and medium scale producers; however, it also poses specific challenges related to the management of abiotic factors, particularly water availability (Gruda, 2019).

Among the key agrotechnical practices in seedling production, irrigation plays a decisive role in germination, growth, root system development, and overall seedling quality (Taiz et al., 2015). Irrigation regimes directly affect plant water status, nutrient uptake, and morphological traits, while inadequate water management may result in poor root development, excessive shoot elongation, or reduced adaptive capacity following transplanting (Rizzo et al., 2018; Chaves et al., 2009). Numerous studies have demonstrated that moderate water deficit during the nursery stage may promote the development of more compact seedlings with a well-developed root system and enhanced tolerance to post-transplant stress (Sánchez-Blanco et al., 2014; Gruda et al., 2018).

In the context of pronounced climate change, manifested in the South Banat region through uneven precipitation distribution and increasingly frequent drought periods, the optimization of irrigation regimes in open cold frames has become particularly important (IPCC, 2021). Although numerous studies have investigated the effects of water regimes on the growth and development of vegetable and ornamental crops, data related to the seedling production of medicinal plants, and particularly *M. vulgare*, under open cold frame conditions remain very limited (Acimović et al., 2020).

Therefore, the aim of this study was to evaluate the effects of different irrigation regimes on the growth, development, and quality of *M. vulgare* L. seedlings produced in open cold frames under the agroecological conditions of the South Banat region, and to identify an optimal irrigation regime that enables the production of high-quality planting material while ensuring rational water use and improved plant resilience to environmental stress.

MATERIAL AND METHODS

Experimental field

The experiment was conducted at the experimental field of the Institute for Medicinal Plant Research “Dr Josif Pančić” in Pančevo, Serbia. Climatic conditions during the experimental period, from late July to early November 2025, were monitored using an automatic data logger (Haxo-8). During the seedling production period, the average air temperature was 20.3 °C, while the total precipitation amounted to 82 mm. Such a low precipitation level indicates pronounced water deficit, despite favorable thermal conditions for seedling growth and development, thus emphasizing the importance of the applied irrigation regimes. The total duration of the experiment was approximately 125 days, from sowing (28 July 2025) to transplanting in the open field (30 November 2025).

The soil at the experimental site, in the surface horizon (0–30 cm), was classified as silty clay loam, with moderate fertility and favorable water–physical properties. Soil reaction was slightly acidic, with pH (H₂O) values ranging from 6.1 to 6.5. Humus content ranged between 3.3 and 3.8%, while total nitrogen content was 0.20–0.23%, with a favorable C/N ratio of 9.9–10.8. The soil was poorly supplied with available phosphorus (2.6–5.9 mg 100 g⁻¹ soil), whereas available potassium content was at a moderate level (10.1–10.7 mg 100 g⁻¹ soil). The cation exchange capacity ranged from 26 to 30 me 100 g⁻¹ soil, with a high base saturation exceeding 90%. Soil physical properties were characterized by total porosity ranging from 41 to 48%, bulk density between 1.37 and 1.57 g cm⁻³, and field water capacity of 30–33 vol.%, indicating moderate water retention capacity.

Experimental Design and Irrigation Regimes (Treatments)

Prior to sowing, the soil was prepared by removing weeds, followed by mechanical tillage and leveling. Open nursery beds (cold beds) measuring 20×1 m were formed, and the irrigation system was installed (Figure 1). The experiment was established as a single-factor trial, where the factor under investigation was the irrigation regime.

Each irrigation regime represented one experimental treatment, and measurements were performed on three representative subsamples within each treatment in order to evaluate seedling emergence and morphological characteristics.



Figure 1. Experimental plots showing different treatments in the field trial

Sowing of *M. vulgare* L. was performed on 28 July 2025 in shallow rows at a depth of 1–2 cm. The sowing rate was 10 g m^{-2} , which is higher than previously reported in the literature (Kišgeci, 2008; Acimović et al., 2020), due to the seed usability value of 60%, in order to ensure adequate plant density and uniform seedling emergence. After sowing, the seeds were lightly covered with finely crumbled soil to ensure proper soil–seed contact and uniform germination conditions.

Irrigation regimes were defined according to the soil water–physical properties (field water capacity 30–33 vol.%, total porosity 41–48%, bulk density $1.37\text{--}1.57 \text{ g cm}^{-3}$) and the precipitation deficit recorded during the experimental period. Three experimental treatments were applied:

- T_0 (control): no irrigation ($0 \text{ l m}^{-2} \text{ day}^{-1}$)
- T_1 : 1.5 L m^{-2} per cycle, four cycles during nighttime (total $6 \text{ l m}^{-2} \text{ day}^{-1}$)
- T_2 : 2.5 L m^{-2} per cycle, four cycles during nighttime (total $10 \text{ l m}^{-2} \text{ day}^{-1}$)

Each irrigation cycle lasted 30 minutes and was performed during nighttime in order to reduce evaporative losses and improve water infiltration. The irrigation system was operated using solenoid valves (Hunter PGV 100, Hunter Industries, USA) connected to an automatic irrigation controller, while water distribution was achieved using micro-sprinklers (type 7110) installed along the central axis of each experimental treatment, ensuring uniform water application. Each irrigation regime represented one experimental treatment.

Morphological Analysis and Emergence Monitoring

Prior to transplanting in the open field, a standardized morphological analysis of *M. vulgare* L. seedlings was performed for each experimental treatment ($T_0\text{--}T_2$). Each irrigation regime represented one experimental treatment, and measurements were performed on three representative subsamples within each treatment. Each subsample consisted of 10 randomly selected plants, resulting in a total of 30 measured plants per treatment.

Morphological measurements were conducted 120 days after sowing, immediately prior to transplanting. The following parameters were evaluated: plant height (cm), measured using a ruler;

rosette diameter (cm), measured across the widest part of the plant; number of leaves per plant; and root collar diameter (mm), measured using a digital caliper (accuracy 0.01 mm).

In addition, root length (cm) was measured using a ruler, and fresh root weight (g) was determined using an analytical balance (accuracy 0.001 g).

Seedling emergence was monitored throughout the experimental period, and the total number of emerged seedlings per experimental treatment was recorded. Emergence percentage (%) was calculated as the ratio between the number of emerged seedlings and the total number of viable seeds sown per unit area, expressed as a percentage.

Based on the obtained morphological and emergence data, seedlings were selected for transplanting according to uniformity and vitality criteria, while the remaining plants were retained within the experimental treatments for further growth and development.

The experimental unit was defined as the experimental treatment area (nursery bed) subjected to a specific irrigation regime.

Data Evaluation

The obtained data were processed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). Morphological and emergence parameters were evaluated based on three representative subsamples within each experimental treatment. The results were expressed as mean $\pm$ standard deviation. Differences between treatment means were assessed using Duncan's multiple range test at the significance level of $p \leq 0.05$. Statistical analysis was performed using IBM SPSS Statistics software (Version 26.0, IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

The applied irrigation regimes significantly influenced seedling emergence, stand density, and morphological characteristics of *M. vulgare* L. seedlings (Table 1), confirming that water availability represents one of the most critical environmental factors affecting early plant development. Similar findings have been reported for numerous medicinal and aromatic plant species, where irrigation directly affected germination, plant density, and seedling quality (Raviv and Lieth, 2008; Acimović et al., 2020).

Table 1. Effect of irrigation regime on emergence and morphological characteristics of *M. vulgare* L. seedlings

Treatment	Number of plants (plants m ⁻²)	Emergence (%)	Plant height (cm)	Rosette diameter (cm)	Root length (cm)	Root fresh weight (g)
T ₀	68 $\pm$ 12.2c	34.0 $\pm$ 6.0c	8.4 $\pm$ 0.9a	10.2 $\pm$ 1.1a	22.3 $\pm$ 2.7a	0.34 $\pm$ 0.06a
T ₁	124 $\pm$ 18.9b	62.0 $\pm$ 9.0b	6.9 $\pm$ 0.8b	8.3 $\pm$ 0.9b	16.1 $\pm$ 2.3b	0.26 $\pm$ 0.05b
T ₂	198 $\pm$ 21.1a	99.0 $\pm$ 10.0a	5.6 $\pm$ 0.7c	6.5 $\pm$ 0.8c	11.4 $\pm$ 1.8c	0.19 $\pm$ 0.04c

Values represent mean $\pm$ standard deviation. Different letters indicate significant differences at $p \leq 0.05$.

The lowest number of emerged seedlings was observed in the non-irrigated control treatment (T₀), where only 68 plants m⁻² emerged, corresponding to 34.0% emergence. As a consequence, a sparse, uneven, and poorly developed stand was formed. Water deficiency during germination represents a major limiting factor, since adequate water supply is essential for seed imbibition, activation of enzymatic systems, and mobilization of storage reserves necessary for embryo growth and emergence (Bewley et al., 2013; Taiz et al., 2015). Similar results were reported by Cantliffe et al. (2007), who emphasized that insufficient soil moisture significantly reduces emergence rate and uniformity in nursery production.

Increasing irrigation level significantly improved emergence and stand establishment. The highest emergence rate was recorded in treatment T₂ (198 plants m⁻², 99.0%), resulting in a dense and uniform

stand. Adequate soil moisture improves germination by facilitating metabolic activity and enhancing seed–soil contact (Taiz et al., 2015). Similar positive effects of irrigation on emergence and stand density have been reported in medicinal plants such as *Melissa officinalis*, *Salvia officinalis*, and *Lavandula angustifolia* (Gruda et al., 2018; Acimović et al., 2020).

However, despite the highest emergence, seedlings in treatment T₂ exhibited significantly reduced morphological development, including the lowest plant height (5.6 cm), rosette diameter (6.5 cm), root length (11.4 cm), and root fresh weight (0.19 g). These results indicate that excessive stand density led to increased competition among plants for essential resources such as light, water, nutrients, and space. Increased plant density is known to reduce individual plant growth due to competition stress, which limits photosynthetic efficiency and root development (Larcher, 2003; Poorter et al., 2012). Similar effects of excessive density on seedling morphology were observed in other Lamiaceae species (Raviv and Lieth, 2008).

In contrast, seedlings grown without irrigation (T₀) exhibited the highest individual morphological values, including plant height (8.4 cm), rosette diameter (10.2 cm), root length (22.3 cm), and root fresh weight (0.34 g). This enhanced individual growth can be attributed to lower plant density and reduced competition, allowing each plant access to more resources. Additionally, water deficit conditions often stimulate root system development as an adaptive response, enabling plants to explore larger soil volumes to access water (Kramer and Boyer, 1995; Taiz et al., 2015). Enhanced root development under moderate water stress has also been reported in medicinal plant seedlings (Rouphael et al., 2014).

Nevertheless, from a practical production standpoint, the non-irrigated treatment cannot be considered optimal due to the low emergence rate and insufficient number of seedlings.

The most favorable balance between seedling quantity and quality was achieved under moderate irrigation (T₁). In this treatment, emergence reached 124 plants m⁻² (62.0%), while seedlings exhibited optimal morphological characteristics, including plant height (6.9 cm), root length (16.1 cm), and root fresh weight (0.26 g). In addition, seedlings were uniform, compact, and well-developed, exhibiting balanced shoot–root development and adequate root system formation, which are recognized indicators of transplant quality. High-quality seedlings are characterized by balanced shoot and root development, which ensures successful establishment after transplanting and improved survival rate (Raviv and Lieth, 2008; Gruda et al., 2018).

These findings clearly demonstrate that optimal irrigation does not necessarily correspond to maximum water supply. Instead, moderate irrigation provides optimal conditions for both emergence and seedling quality by balancing water availability and plant density. Excessive irrigation, while improving emergence, negatively affects seedling quality due to increased competition, while insufficient irrigation limits emergence despite improving individual growth.

Overall, moderate irrigation (T₁) can be considered the optimal regime for the production of high-quality *M. vulgare* seedlings under open-field nursery conditions.

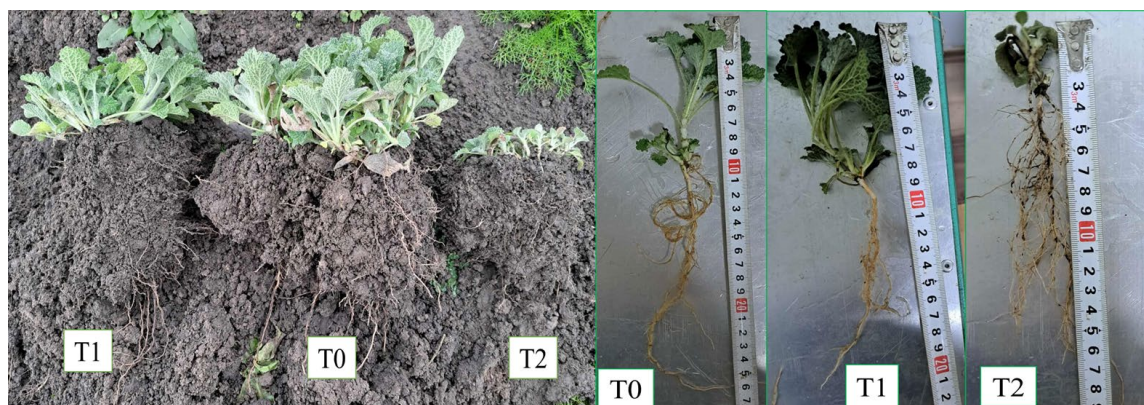


Figure 2. Effect of treatments (T₁ and T₂) on shoot and root morphology of *M. vulgare* L. plants compared to control (T₀)

CONCLUSION

The results of this study demonstrated that irrigation regime had a significant effect on seedling emergence, stand density, and morphological characteristics of *M. vulgare* L. seedlings under open-field nursery conditions. Increasing irrigation level significantly improved seedling emergence and stand formation; however, excessive irrigation resulted in reduced individual seedling quality due to increased plant density and competition. In contrast, the absence of irrigation promoted the development of larger and more robust individual seedlings but resulted in a significantly lower emergence rate and insufficient seedling number for practical production. The most favorable results were achieved under moderate irrigation ($6 \text{ L m}^{-2} \text{ day}^{-1}$), which provided an optimal balance between seedling emergence and morphological quality. Seedlings produced under this regime exhibited uniform growth, well-developed root systems, and favorable morphological characteristics, ensuring high transplanting potential. Based on the obtained results, moderate irrigation can be recommended as the optimal regime for the production of high-quality *M. vulgare* L. seedlings in open-field nursery production systems.

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REFERENCES

- Acimović, M., Jeremić, K., Salaj, N., Gavarić, N., Kiproviski, B., Sikora, V., & Zeremski, T. (2020). *Marrubium vulgare* L.: A phytochemical and pharmacological overview. *Molecules*, 25(12), 2898. <https://doi.org/10.3390/molecules25122898>
- Acimović, M., Kiproviski, B., Sikora, V., Brdar-Jokanović, M., & Zeremski, T. (2020). Production and cultivation of medicinal and aromatic plants in Serbia. *Industrial Crops and Products*, 154, 112693.
- Basar, S., Demirci, B., & Demirci, F. (2013). Chemical composition and biological activity of *Marrubium* species. *Industrial Crops and Products*, 43, 402–407.
- Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., & Nonogaki, H. (2013). *Seeds: Physiology of development, germination and dormancy*. Springer.
- Cantliffe, D. J., Hochmuth, G. J., & Secker, I. (2007). Transplant production systems for vegetable crops. *Horticultural Reviews*, 33, 1–52.
- Chaves, M. M., Flexas, J., & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Annals of Botany*, 103(4), 551–560. <https://doi.org/10.1093/aob/mcn125>
- Gruda, N. (2019). Increasing sustainability of growing media constituents and stand-alone substrates in soilless culture systems. *Agronomy*, 9(6), 298. <https://doi.org/10.3390/agronomy9060298>
- Gruda, N., Bisbis, M., & Tanny, J. (2018). Influence of climate change on protected cultivation: Impacts and sustainable adaptation strategies. *Journal of Cleaner Production*, 183, 890–902. <https://doi.org/10.1016/j.jclepro.2019.03.210>
- Gurčik, L., Mihálik, D., & Repčák, M. (2005). Economic aspects of medicinal plant production. *Agricultural Economics*, 51(10), 459–465.
- IPCC. (2021). *Climate change 2021: The physical science basis*. Cambridge University Press.
- Kišgeci, J. (2008). *Lekovito bilje – gajenje, sakupljanje i prerada*. Nolit, Beograd.
- Kramer, P. J., & Boyer, J. S. (1995). *Water relations of plants and soils*. Academic Press.
- Larcher, W. (2003). *Physiological plant ecology*. Springer.
- Letchamo, W., Ward, W., & Heard, B. (2006). Quality evaluation of medicinal plant transplants. *Journal of Herbs, Spices & Medicinal Plants*, 12(3), 29–41.

- Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytologist*, 193(1), 30–50. <https://doi.org/10.1111/j.1469-8137.2011.03952.x>
- Raviv, M., & Lieth, J. H. (2008). *Soilless culture: Theory and practice*. Elsevier.
- Rizzo, R., Raimondi, G., & De Pascale, S. (2018). Water management strategies to improve transplant quality. *Scientia Horticulturae*, 233, 347–355.
- Rouphael, Y., Cardarelli, M., Schwarz, D., Franken, P., & Colla, G. (2014). Effects of drought on plant growth and root development. *Plant Physiology and Biochemistry*, 80, 54–62.
- Sánchez-Blanco, M. J., Álvarez, S., Ortuño, M. F., & Ruiz-Sánchez, M. C. (2014). Root system response to drought and recovery in young plants. *Plant Physiology and Biochemistry*, 74, 123–131.
- Shakeri, A., Sahebkar, A., & Javadi, B. (2016). *Melissa officinalis* L. – A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, 188, 204–228. <https://doi.org/10.1016/j.jep.2016.05.010>
- Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant physiology and development*. Sinauer Associates.

Evaluation of UAV-based multispectral vegetation indices for disease and stress detection in vineyards

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Abstract

In areas with a long-standing tradition of wine production, vineyard management increasingly requires the application of precise and data driven agricultural practices. The adoption of advanced technologies in viticulture has enabled the collection of large amounts of data, supporting early detection of potential problems and providing valuable insights into crop condition and overall vineyard status. The objective of this paper is to analyze the potential of multispectral imaging for vineyard monitoring and to examine its role in improving decision making in modern viticulture. The paper provides an overview of the principles of multispectral sensing and the use of vegetation indices, particularly NDVI and NDRE, for assessing grapevine condition. Based on the reviewed literature, multispectral imaging enables precise monitoring of grapevine status, including the detection of stress, diseases, and pests, as well as the assessment of vine growth, development, and spatial variability within vineyards. Furthermore, its integration with UAV systems allows for efficient data acquisition and high-resolution analysis at the field level. Although certain limitations related to environmental conditions, data interpretation, and sensor calibration remain, multispectral technologies have significant potential to optimize grape production and support more sustainable and resource efficient vineyard management.

Keywords: Canopy monitoring, NDVI, NDRE, crop health assessment, site-specific management, UAV technology

INTRODUCTION

Viticulture represents an important sector of agriculture with significant economic, environmental, and cultural relevance (Polge de Combret-Champart et al., 2013; Cataldo et al 2021). The wine industry is directly dependent on grape production, which plays a crucial role in the economic development of many regions worldwide. In areas with a long-standing tradition of wine production, vineyard management requires increasingly precise agricultural practices. As in other agricultural systems, the adoption of advanced technologies in viticulture has enabled the collection of large amounts of data that can support the early detection of potential problems and provide valuable information on crop condition and overall vineyard status (Marina Stević et al., 2025).

Remote sensing enables the monitoring of vineyard growth and vine condition using satellite imagery and unmanned aerial vehicles (UAVs). Multispectral imaging represents a modern technology that employs specialized sensors and cameras to analyze plant surfaces across different wavelengths of the electromagnetic spectrum (Deng et al., 2018). This approach allows for more precise monitoring of grapevine condition, providing a reliable basis for timely and informed management decisions. Multispectral sensors acquire data in several spectral bands, including visible wavelengths (red, green, and blue) and near-infrared regions, which provide important information about plant health, vegetation vigor, and resource requirements. The differentiation of these spectral regions enables the detection of specific plant characteristics that are not visible to the naked eye. The objective of this paper is to analyze the

potential of multispectral imaging for vineyard monitoring and to discuss its role in improving decision-making in modern viticulture.

FUNDAMENTALS OF MULTISPECTRAL IMAGING

Multispectral monitoring of vineyards can be performed using several approaches, including ground-based handheld sensors, aerial platforms such as UAVs, and satellite imagery. Each of these approaches has specific advantages and limitations depending on spatial coverage, data resolution, and operational efficiency. Among them, UAV-based data acquisition represents one of the most suitable solutions for vineyard monitoring, as it enables rapid and efficient data collection at the local scale. UAVs can operate at low altitudes above the vineyard canopy and capture high-resolution imagery of specific vine areas, providing detailed information on plant condition (Song et al., 2022; Zhu et al., 2022). In contrast, satellite imagery offers a broader perspective for assessing large vineyard areas, although typically with lower spatial resolution. Ground-based handheld devices, on the other hand, can provide highly detailed measurements but require considerably more time when monitoring larger vineyard areas (Cai et al., 2021).

Multispectral imaging can also contribute to more efficient management of plant diseases and pests. Early detection of disease symptoms or insect infestations, which may be identified through changes in the spectral response of plants, enables vineyard managers to implement timely protection measures (Pajić et al., 2019; Peng et al., 2022). Such an approach supports targeted crop protection practices, allowing the application of chemical treatments only in affected areas and thereby reducing the overall use of agrochemicals.

Processing data obtained from multispectral imagery enables the calculation of various vegetation indices. These indices are widely used for both qualitative and quantitative assessment of vegetation and for monitoring crop growth dynamics (Xu and Su, 2017; Lazarević et al., 2025). Among the most commonly used spectral indices in both scientific research and practical applications are the Normalized Difference Vegetation Index (NDVI), the Normalized Difference Red Edge Index (NDRE), and the Soil Adjusted Vegetation Index (SAVI), along with numerous other indices designed to assess different aspects of plant condition.

The NDVI is the most widely used, as it can be calculated from sensors that capture data in the visible and near-infrared (NIR) spectral bands. NDVI is effective for assessing vegetation status and various quantitative characteristics of plant growth (Huang et al., 2021). In comparison with NDVI, the NDRE is based on the red-edge spectral region, making it less susceptible to saturation effects and more sensitive to variations in chlorophyll content within vegetation (Jorge et al., 2019). To reduce the influence of soil background on vegetation analysis, the SAVI is commonly used. This index was developed to minimize the effect of soil reflectance in vegetation index calculations (Vani and Mandla, 2017).

APPLICATIONS IN VINEYARDS

Multispectral cameras are widely used in vineyards for precise monitoring of grapevine condition, detection of diseases and pests, and assessment of vine growth and development, as well as for vineyard zoning. Recent studies in viticulture employing vegetation indices have demonstrated strong relationships between vegetation index (VI) values and specific grapevine characteristics, including vine vigor, yield, and plant health status. Among these parameters, vine vigor has proven to be a particularly useful indicator for identifying qualitative zones within vineyards. Vineyard zoning enables more informed management decisions during key stages of grape production. Consequently, it supports practices such as selective harvesting based on grape quality (Bramley, 2010).

Lee et al. (2025) highlighted one of the key advantages of multispectral imaging in vineyard monitoring. Their study demonstrated that the NDVI can be used to identify areas within vineyards exhibiting signs of stress due to diseases or unfavorable environmental conditions. The authors also concluded that UAV-based systems could become standard tools in vineyard management in the future.

Another study demonstrated that the integration of thermal, multispectral, and three-dimensional (3D) imagery enables the generation of detailed vineyard maps, facilitating the identification of specific areas that require additional irrigation. This approach contributes to reduced water losses and improved irrigation efficiency (Burchard-Levine et al., 2024).

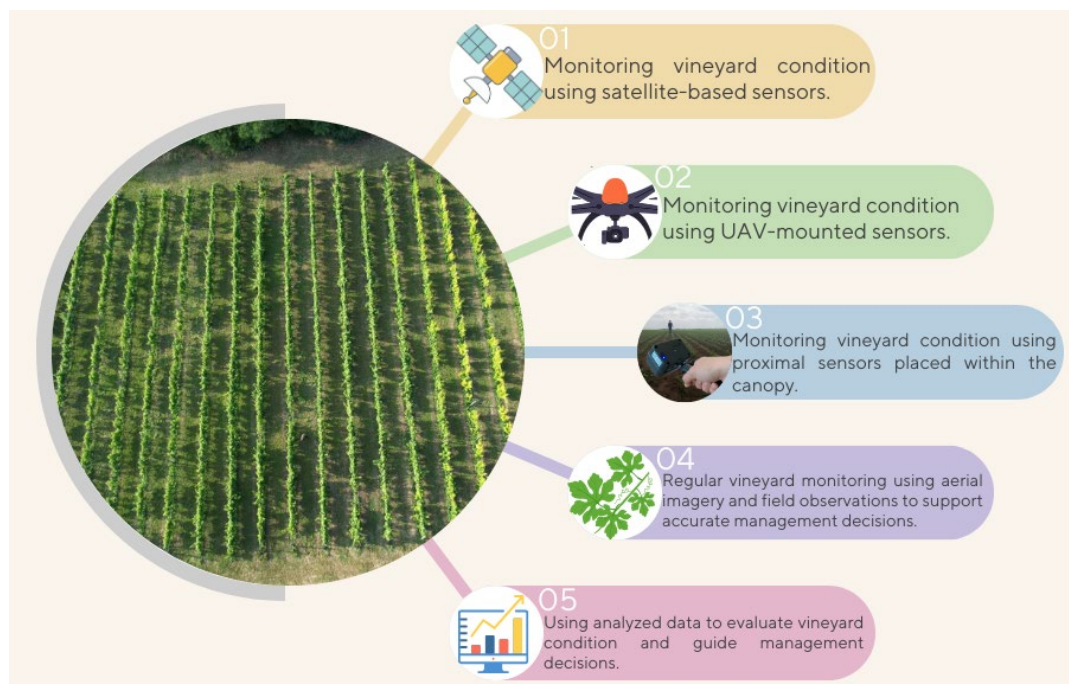


Figure 1. Overview of vineyard monitoring approaches using satellite imagery, UAV platforms, and proximal sensors

Considering the ongoing impacts of global climate change, which influence precipitation patterns, temperature regimes, and other environmental factors affecting grapevine health, multispectral imaging is becoming an increasingly important tool for adapting viticulture to emerging challenges. Vineyard managers who adopt these technologies are better equipped to respond to such changes and to maintain sustainable and efficient grape production.

One of the main challenges associated with the implementation of multispectral imaging in vineyards is the high initial cost of equipment acquisition, including UAV platforms and data processing software, as well as the need for training personnel in data analysis and interpretation. However, with ongoing technological advancements, the cost of UAVs, sensors, and software solutions is gradually decreasing, making these technologies increasingly accessible. Furthermore, investments in multispectral imaging can lead to significant long-term benefits, including reduced production costs and improved yields, making this technology economically viable for vineyard management.

CONCLUSION

Multispectral imaging of vineyards represents an important step toward the modernization of agricultural production, particularly in viticulture. As highlighted throughout this study, the application of this technology enables precise monitoring of grapevine condition and supports more informed vineyard management. However, several challenges still limit its broader adoption, including the high cost of technology, the need for specialized knowledge in data interpretation, and potential technical issues related to equipment operation. Despite these limitations, multispectral imaging is expected to become increasingly important in vineyard management in the future. Advances in data analysis systems, decreasing equipment costs, and integration with emerging technologies such as artificial intelligence may further enhance the efficiency and sustainability of viticulture practices. With continuous technological development, multispectral imaging is likely to play a key role in optimizing grape production while contributing to more sustainable and resource-efficient vineyard management.

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REFERENCES

- Bramley, R. G. V. (2010). Precision viticulture: Managing vineyard variability for improved quality outcomes. In A. G. Reynolds (Ed.), *Managing Wine Quality* (pp. 445–480). Woodhead Publishing.
- Burchard-Levine, V., Guerra, J. G., Borra-Serrano, I., Nieto, H., Mesías-Ruiz, G., Dorado, J., & Peña, J. M. (2024). Evaluating the utility of combining high resolution thermal, multispectral and 3D imagery from unmanned aerial vehicles to monitor water stress in vineyards. *Precision Agriculture*, 25(5), 2447–2476. <https://doi.org/10.1007/s11119-024-10179-0>
- Cai, Z., Liu, Z., & Kou, L. (2021). Reliable UAV monitoring system using deep learning approaches. *IEEE Transactions on Reliability*, 71(2), 973–983. <https://doi.org/10.1109/TR.2021.3119068>
- Cataldo, E., Fucile, M., & Mattii, G. B. (2021). Soil management, sustainable strategies and approaches to improve the quality of modern viticulture: A review. *Agronomy*, 11(11), 2359. <https://doi.org/10.3390/agronomy11112359>
- Deng, L., Mao, Z., Li, X., Hu, Z., Duan, F., & Yan, Y. (2018). UAV-based multispectral remote sensing for precision agriculture: A comparison between different cameras. *Journal of Photogrammetry and Remote Sensing*, 146, 124–136. <https://doi.org/10.1016/j.isprsjprs.2018.09.008>
- Huang, S., Tang, L., Hupy, J. P., Wang, Y., & Shao, G. (2021). A commentary review on the use of normalized difference vegetation index (NDVI) in the era of popular remote sensing. *Journal of Forestry Research*, 32(1), 1–6. <https://doi.org/10.1007/s11676-020-01155-1>
- Jorge, J., Vallbé, M., & Soler, J. A. (2019). Detection of irrigation inhomogeneities in an olive grove using the NDRE vegetation index obtained from UAV images. *European Journal of Remote Sensing*, 52(1), 169–177. <https://doi.org/10.1080/22797254.2019.1572459>
- Lazarević, N., Lazarević, A., Bošković, B., Dražić, M., Gligorević, K., Buđen, M., & Pajić, M. (2025). Multispectral assessment of UAV and field sprayer chemical crop protection in wheat. *Poljoprivredna Tehnika*, 50(3), 75–84. <https://doi.org/10.5937/POLJTEH2503075L>
- Lee, L., Reynolds, A., Dorin, B., & Shemrock, A. (2025). Potential of a remotely piloted aircraft system with multispectral and thermal sensors to monitor vineyard characteristics for precision viticulture. *Plants*, 14(1), 137. <https://doi.org/10.3390/plants14010137>
- Pajić, M., Oparnica, S., Oljača, M., Gligorević, K., Dražić, M., Zlatanović, I., & Bošković, B. (2019). Mapping treatments of chemical protection in barley production by using precision agriculture technology. *Poljoprivredna Tehnika*, 44(2), 47–55. <http://doi.org/10.5937/PoljTeh1902047P>
- Peng, Y., Dallas, M. M., Ascencio-Ibáñez, J. T., Hoyer, J. S., Legg, J., Hanley-Bowdoin, L., & Yin, H. (2022). Early detection of plant virus infection using multispectral imaging and spatial-spectral machine learning. *Scientific Reports*, 12(1), 3113. <https://doi.org/10.1038/s41598-022-06372-8>
- Polge de Combret-Champart, L., Guilpart, N., Merot, A., Capillon, A., & Gary, C. (2013). Determinants of the degradation of soil structure in vineyards with a view to conversion to organic farming. *Soil Use and Management*, 29(4), 557–566. <https://doi.org/10.1111/sum.12071>
- Song, X., Wu, F., Lu, X., Yang, T., Ju, C., Sun, C., & Liu, T. (2022). The classification of farming progress in rice–wheat rotation fields based on UAV RGB images and the regional mean model. *Agriculture*, 12(2), 124. <https://doi.org/10.3390/agriculture12020124>
- Stević, I. M., Bošković, B., Živojinović, D., Stevanović, N., Stanković, N., & Buđen, M. (2025). Comparison of NDVI index in vine with the application of different chemical treatments. In *Proceedings of the V International Scientific Conference Sustainable Agriculture and Rural Development* (pp. 585–592). Institute of Agricultural Economics.
- Vani, V., & Mandla, V. R. (2017). Comparative study of NDVI and SAVI vegetation indices in Anantapur district semi-arid areas. *International Journal of Civil Engineering and Technology*, 8(4), 559–566.
- Xue, J., & Su, B. (2017). Significant remote sensing vegetation indices: A review of developments and applications. *Journal of Sensors*, 1353691. <https://doi.org/10.1155/2017/1353691>
- Zhu, W., Feng, Z., Dai, S., Zhang, P., & Wei, X. (2022). Using UAV multispectral remote sensing with appropriate spatial resolution and machine learning to monitor wheat scab. *Agriculture*, 12(11), 1785. <https://doi.org/10.3390/agriculture12111785>

Root-associated beneficial microorganisms enhance nutrient efficiency–related plant responses and fruit functional quality in sustainable raspberry production

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Abstract

Enhancing nutrient use efficiency while preserving fruit functional quality represents a critical bottleneck in the transition toward sustainable raspberry production systems. Root-associated beneficial microorganisms are increasingly recognized as key drivers of rhizosphere processes that regulate nutrient cycling and influence plant metabolic responses. This study evaluated the field-level integration of a consortium of selected root-associated beneficial microorganisms, formulated as a microbial-enriched vermicompost extract developed at the Fruit Research Institute Čačak, Serbia, within contrasting fertilization strategies. The experiment compared conventional mineral fertilization with two integrated fertilization systems combining mineral fertilization with microbial-based inputs (microbial-enriched vermicompost extract or humus-based fertilizer) and zeolite, together with a microbial-enriched vermicompost extract treatment applied independently and an unfertilized control. The biologically supported mineral systems, particularly the microbial-enriched system, significantly increased the number of fruiting laterals per florican and fruits per fruiting lateral, fruit weight, and soluble solids content compared with mineral fertilization alone ($p < 0.05$). Treatments involving the microbial consortium, either applied independently or integrated with mineral fertilization, also enhanced total phenolic content, anthocyanins, and antioxidant activity (DPPH and ABTS assays), indicating improved regulation of secondary metabolism. The most pronounced effects were observed when microbial consortia were combined with mineral fertilization, suggesting synergistic interactions between microbial functionality and nutrient inputs. Collectively, the results highlight the agronomic relevance of root-associated beneficial microorganisms in enhancing nutrient use efficiency and fruit functional quality, supporting their integration into sustainable raspberry production systems.

Keywords: root-associated beneficial microorganisms, fertilization strategy, nutrient use efficiency, raspberry, fruit quality

INTRODUCTION

Modern berry production systems face increasing pressure to sustain high productivity while reducing the environmental impacts associated with intensive fertilization and inefficient nutrient management. Conventional fertilization strategies dominated by mineral inputs often result in nutrient losses, soil degradation, and disruption of soil biological processes, ultimately compromising long-term agroecosystem sustainability (Tilman et al., 2002; Zhang et al., 2015). These challenges are particularly pronounced in horticultural crops characterized by high nutrient demand and intensive management practices.

In recent years, root-associated beneficial microorganisms have emerged as key biological drivers of sustainable agricultural intensification. These microorganisms contribute to plant nutrition through multiple mechanisms, including biological nitrogen fixation, phosphorus solubilization, siderophore production, and stimulation of root system development, thereby improving nutrient availability and plant uptake efficiency (Adesemoye & Kloepper, 2009; Richardson et al., 2009; Backer et al., 2018). Increasing attention has also been given to plant–microbiome interactions as key determinants of plant health and productivity (Trivedi et al., 2020).

Beyond their role in nutrient acquisition, beneficial microorganisms function as regulators of plant physiological processes. Microbial interactions in the rhizosphere can influence hormonal signaling pathways, enhance plant tolerance to environmental stresses, and modulate metabolic pathways associated with plant growth and defense responses (Rouphael et al., 2017; Ali et al., 2021).

Compost- and vermicompost-derived microbial formulations represent a promising strategy for introducing beneficial microorganisms into agricultural soils. Microbial-enriched vermicompost extracts contain diverse microbial communities together with soluble organic compounds capable of stimulating microbial establishment and rhizosphere activity (Arancon et al., 2006; Joshi et al., 2015).

In berry crops such as raspberry (*Rubus idaeus* L.), fruit quality traits — including soluble solids, phenolic compounds, anthocyanins, and antioxidant activity — represent key determinants of both market value and nutritional quality. Increasing evidence suggests that rhizosphere microbial activity can influence plant secondary metabolism and thereby affect fruit biochemical composition (García-Seco et al., 2015; Wu et al., 2022).

Despite growing interest in microbial-based inputs, field studies evaluating their integration with conventional fertilization strategies remain limited. Understanding how microbial consortia interact with mineral nutrient supply is therefore essential for developing practical fertilization strategies capable of improving both crop productivity and fruit functional quality.

Therefore, the aim of this study was to evaluate the effects of integrating microbial-enriched vermicompost extract into fertilization strategies on vegetative growth, fruit quality, and antioxidant-related parameters in raspberry under field conditions.

MATERIAL AND METHODS

Experimental site and design

The field experiment was conducted during the 2024 growing season in a commercial raspberry (*Rubus idaeus* L.) plantation located in Grab village near Čačak, Serbia (43.8249° N, 20.2849° E). The study evaluated the influence of root-associated beneficial microorganisms integrated into different fertilization strategies. The experiment was established in a randomized complete block design with five treatments and three replications. Each replicate consisted of ten plants. Five fertilization regimes were evaluated: conventional mineral fertilization (T1, CMnF); mineral fertilization supplemented with microbial-enriched vermicompost extract and zeolite (T2, CMnF + MeVCT + Zeo); mineral fertilization combined with humus-based fertilizer and zeolite (T3, CMnF + HV + Zeo); microbial-enriched vermicompost extract applied independently (T4, MeVCT); and an unfertilized control (T5).

Fertilization treatments and application

The microbial-enriched vermicompost extract was developed at the Fruit Research Institute, Čačak, as described by Pešaković et al. (2023). Applications were performed during key phenological stages of raspberry development, including bud break, cane elongation, flowering, and fruit set stage. Mineral fertilization followed standard commercial practice and was applied at the beginning of vegetative growth and during active primocane development.

Yield-related traits

At full bloom, the number of fruiting laterals per florican and fruits per fruiting lateral were recorded. Measurements were conducted on all plants within each replicate.

Fruit quality and biochemical analyses

Ripe fruits were harvested at commercial maturity and stored at 4 °C prior to analysis. Twenty fruits per repetition were randomly selected in one treatment (125 fruits in total). Fruits were picked from different parts of the floricanes to avoid fruit position effect (Karaklajić Stajić et al., 2023). Berries were harvested at a red-ripe stage of maturity when they were suitable for the fresh market. Average fruit samples were taken from fruit harvested at the beginning, middle, and end of the harvest period and used for market-related and antioxidant-related traits.

Fruit weight was determined using a digital balance, while soluble solids content (SSC) was measured using a digital refractometer. Total phenolic content was determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents per 100 g fresh weight (Singleton et al., 1999). Total anthocyanins were determined using the pH differential method (Giusti & Wrolstad, 2001). Antioxidant activity was evaluated using DPPH and ABTS radical scavenging assays (Brand-Williams et al., 1995; Re et al., 1999).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA). Mean comparisons were performed using Tukey's HSD test at a significance level of $p < 0.05$ using IBM SPSS Statistics software.

RESULTS

Yield-related traits

Integrated fertilization strategies supported by root-associated beneficial microorganisms significantly influenced raspberry yield-related traits (Table 1). Treatments combining mineral fertilization with microbial inputs (T2 and T3) produced a significantly higher number of fruiting laterals per floricanes and fruits per fruiting lateral compared with conventional mineral fertilization alone (T1) and the unfertilized control (T5) ($p < 0.05$).

The highest values were observed in T2 (20.3 fruiting laterals per floricanes and 3.8 fruits per fruiting lateral) and T3 (19.8 and 3.7 respectively), which did not differ significantly from each other. The treatment based solely on microbial-enriched vermicompost extract (T4) also resulted in improved yield-related traits compared with T1 and the control, although the increase was less pronounced.

Conversely, plants grown under mineral fertilization alone (T1) showed yield-related traits comparable to those observed in the unfertilized control (T5). Overall, the results demonstrate that fertilization strategies supported by beneficial microorganisms promote improved yield-related traits in raspberry plants.

Table 1. Effect of integrated fertilization strategies on yield-related traits of raspberry

Treatment	Fruiting laterals per floricanes	Fruits per fruiting lateral
T1	16.1 ± 1.8 ^c	3.0 ± 0.2 ^c
T2	20.3 ± 2.1 ^a	3.8 ± 0.5 ^a
T3	19.8 ± 1.9 ^a	3.7 ± 0.4 ^a
T4	18.5 ± 2.3 ^b	3.4 ± 0.3 ^b
T5	15.4 ± 2.0 ^c	2.9 ± 0.3 ^c

Values represent mean ± standard deviation (n = 3). Different letters within columns indicate significant differences according to Tukey's HSD test ($p < 0.05$) following one-way ANOVA.

Market-related traits

Fruit weight and soluble solids content (SSC) responded positively to fertilization strategies supported by root-associated microorganisms (Table 2). The highest fruit weight was recorded in T2 (5.10 g) and T4 (5.05 g), both significantly higher than values observed in T1 and T5 ($p < 0.05$). Treatment T3 also improved fruit weight (4.85 g), although to a slightly lesser extent.

A similar trend was observed for SSC. The highest values were measured in T2 (11.2 °Brix) and T4 (11.1 °Brix), followed by T3 (10.8 °Brix), while mineral fertilization alone (T1) and the control (T5) showed significantly lower soluble solids content.

These results demonstrate that fertilization strategies incorporating microbial inputs contribute to improved market-related fruit quality in raspberry.

Table 2. Effect of integrated fertilization strategies on market-related traits of raspberry

Treatment	Fruit weight (g)	SSC (°Brix)
T1	4.20 ± 0.10 ^c	9.9 ± 0.3 ^c
T2	5.10 ± 0.20 ^a	11.2 ± 0.3 ^a
T3	4.85 ± 0.18 ^b	10.8 ± 0.3 ^b
T4	5.05 ± 0.25 ^a	11.1 ± 0.4 ^a
T5	4.40 ± 0.15 ^c	10.1 ± 0.2 ^c

Values represent mean ± standard deviation (n = 3). Different letters within columns indicate significant differences according to Tukey's HSD test ($p < 0.05$) following one-way ANOVA.

Antioxidant-related traits

Fertilization strategies also significantly influenced antioxidant-related fruit quality parameters (Table 3). Fruits obtained from integrated fertilization treatments exhibited higher concentrations of total phenolics, anthocyanins, and antioxidant activity compared with mineral fertilization alone and the control ($p < 0.05$).

The highest total phenolic content was recorded in T2 (195.0 mg GAE/100 g FW) and T4 (192.6 mg GAE/100 g FW), followed by T1 and T3, while the lowest value was observed in the unfertilized control (T5). A similar pattern was observed for anthocyanins and antioxidant activity. Treatments T2 and T4 produced the highest anthocyanin concentrations and antioxidant activity measured by DPPH and ABTS assays. In contrast, the lowest antioxidant values were recorded in T5 and T3, depending on the parameter evaluated.

Overall, the results indicate that fertilization strategies incorporating microbial inputs can substantially improve antioxidant-related fruit quality traits in raspberry.

Table 3. Effect of integrated fertilization strategies on antioxidant-related traits of raspberry

Treatment	Total phenolics (mg GAE/100 g FW)	Anthocyanins (mg C3G/g FW)	DPPH (%)	ABTS (mmol TE/100 g FW)
T1	186.6 ± 6.0 ^b	57.5 ± 2.0 ^b	16.2 ± 1.1 ^c	1.09 ± 0.03 ^c
T2	195.0 ± 5.2 ^a	60.1 ± 1.5 ^a	21.5 ± 0.9 ^a	1.27 ± 0.04 ^a
T3	182.7 ± 5.4 ^c	52.4 ± 1.7 ^c	12.6 ± 0.8 ^d	0.98 ± 0.02 ^d
T4	192.6 ± 4.8 ^a	61.6 ± 1.8 ^a	19.3 ± 1.0 ^b	1.14 ± 0.05 ^b
T5	175.7 ± 4.9 ^d	50.3 ± 1.9 ^d	10.3 ± 0.7 ^d	0.93 ± 0.01 ^d

Values represent mean ± standard deviation (n = 3). Different letters within columns indicate significant differences according to Tukey's HSD test ($p < 0.05$) following one-way ANOVA.

DISCUSSION

The present study demonstrates that integrating root-associated beneficial microorganisms into fertilization strategies can significantly enhance reproductive performance and fruit biochemical quality in raspberry production systems. As microbial composition and activity were not directly measured in this study, these interpretations are based on established mechanisms reported in the literature. The superior performance observed in treatments combining microbial inputs with mineral fertilization indicates that rhizosphere-mediated biological processes play an important role in improving nutrient use efficiency and regulating plant physiological responses.

Enhanced yield-related traits observed in the integrated fertilization treatments may be associated with improved nutrient acquisition, as reported for beneficial microorganisms in previous studies. Plant growth-promoting rhizobacteria, particularly members of the genera *Bacillus* and *Pseudomonas*, are widely recognized for their ability to facilitate biological nitrogen fixation, solubilize phosphorus, produce siderophores, and stimulate root system development, thereby increasing nutrient availability and plant uptake efficiency (Adesemoye & Kloepper, 2009; Richardson et al., 2009; Backer et al., 2018). Through these mechanisms, microbial inoculants have been reported to enhance and improve plant access to essential macro- and micronutrients.

While improved nutrient acquisition primarily affects the number of fruiting laterals per florican and fruits per fruiting lateral, enhanced plant physiological activity may also influence fruit development and quality. The increases in fruit weight and soluble solids content observed in the integrated treatments indicate improved assimilate production and allocation during fruit development. Similar improvements in crop performance under reduced mineral fertilization combined with plant growth-promoting microorganisms have been reported in other horticultural systems (Tomić et al., 2018; Abdel Salama et al., 2022). Microbial biostimulants can stimulate plant physiological activity, including photosynthetic efficiency and nutrient assimilation, thereby supporting enhanced fruit productivity and quality (Rouphael et al., 2017; Bhardwaj et al., 2014).

Beyond agronomic responses, microbially supported fertilization significantly enhanced total phenolic content, anthocyanins, and antioxidant activity in raspberry fruits. Such biochemical responses suggest that rhizosphere microbial activity may influence plant secondary metabolism, particularly pathways associated with phenolic compound biosynthesis (Tomić et al., 2018; Pešaković et al., 2023; Tomić et al., 2025). Microbial signaling interactions and improved nutrient availability can stimulate the phenylpropanoid pathway, potentially resulting in increased accumulation of antioxidant compounds in fruits as suggested in previous studies (García-Seco et al., 2015; Wu et al., 2022). Considering that berries are widely recognized as functional foods rich in bioactive compounds, improvements in antioxidant-related traits represent an important added value for both nutritional quality and market competitiveness (Giampieri et al., 2014).

Fertilization practices are also known to influence the structure and functional potential of rhizosphere microbial communities. Intensive mineral fertilization has been associated with reduced microbial diversity and altered microbial interaction networks, potentially affecting ecosystem stability and nutrient cycling processes (Banerjee et al., 2019). In contrast, fertilization systems supported by biological inputs may promote more active microbial communities and improved soil biological functionality (Pešaković et al., 2013; Lori et al., 2017; Compant et al., 2019).

Overall, the results indicate that fertilization strategies combining mineral fertilization with microbial inputs can improve both agronomic performance and fruit biochemical quality in raspberry production systems. In addition to enhancing yield-related traits, these treatments were associated with increased concentrations of antioxidant compounds in fruits, suggesting that microbial amendments may influence plant metabolic processes linked to secondary metabolite accumulation. These findings support the growing evidence that biologically supported fertilization systems represent a promising approach for improving crop productivity and fruit quality within more sustainable horticultural production systems.

CONCLUSION

This study demonstrates that fertilization strategies integrating root-associated beneficial microorganisms can significantly improve both agronomic performance and fruit biochemical quality in raspberry production systems. The biologically supported mineral fertilization, particularly the treatment combining mineral fertilization with microbial-enriched vermicompost extract and zeolite, consistently enhanced yield-related traits and fruit quality attributes, including antioxidant-related parameters compared with conventional mineral fertilization alone.

The observed improvements suggest that beneficial microbial consortia may contribute to improved nutrient use efficiency while simultaneously modulating plant secondary metabolism associated with phenolic and anthocyanin accumulation. These findings highlight the functional role of rhizosphere microorganisms in mediating plant–soil interactions that influence both yield formation and fruit nutritional quality.

From a broader agronomic perspective, the integration of microbial inputs into fertilization regimes represents a promising strategy for reducing the dependency on intensive mineral fertilization while maintaining high productivity and fruit quality. Such approaches align with emerging sustainability frameworks in horticultural production that emphasize improved nutrient cycling, soil biological activity, and environmentally responsible management practices.

Overall, the findings highlight the agronomic and physiological relevance of integrating beneficial microbial inputs into fertilization regimes, demonstrating their potential to enhance both productivity and fruit biochemical quality in raspberry cultivation.

Further research including direct measurements of microbial activity and nutrient dynamics is required to confirm the underlying mechanisms.

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REFERENCES

- Abdel-Salama, A. M., Youssef, G. H., & Hassan, E. A. (2022). Plant growth-promoting rhizobacteria improve nutrient availability, growth, and productivity of horticultural crops under reduced mineral fertilization. *Journal of Soil Science and Plant Nutrition*, 22, 356–370.
- Adesemoye, A. O., & Kloepper, J. W. (2009). Plant–microbe interactions in enhanced fertilizer-use efficiency. *Applied Microbiology and Biotechnology*, 85, 1–12. <https://doi.org/10.1007/s00253-009-2196-0>
- Ali, S., Charles, T. C., & Glick, B. R. (2021). Plant growth-promoting bacteria and the mitigation of abiotic stress in plants. *Applied Microbiology and Biotechnology*, 105, 2685–2698.
- Arancon, N. Q., Edwards, C. A., Bierman, P., Metzger, J. D., & Lucht, C. (2006). Effects of vermicomposts on growth and marketable fruits of field-grown tomatoes, peppers and strawberries. *Pedobiologia*, 50, 203–212. <https://doi.org/10.1078/0031-4056-00251>
- Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., & Smith, D. L. (2018). Plant growth-promoting rhizobacteria: mechanisms and applications. *Frontiers in Plant Science*, 9, 1473. <https://doi.org/10.3389/fpls.2018.01473>
- Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A., Gatteringer, A., Keller, T., Charles, R., & van der Heijden, M. G. A. (2019). Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. *The ISME Journal*, 13, 1722–1736. <https://doi.org/10.1038/s41396-019-0383-2>

- Bhardwaj, D., Ansari, M. W., Sahoo, R. K., & Tuteja, N. (2014). Biofertilizers function as key players in sustainable agriculture. *Microbial Cell Factories*, 13, 66. <https://doi.org/10.1186/1475-2859-13-66>
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, 28, 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*, 19, 29–37. <https://doi.org/10.1016/j.jare.2019.03.004>
- García-Seco, D., Zhang, Y., Gutierrez-Mañero, F. J., Martín, C., & Ramos-Solano, B. (2015). Application of *Pseudomonas fluorescens* to blackberry under field conditions improves fruit quality by modifying flavonoid metabolism. *Journal of Agricultural and Food Chemistry*, 63, 972–982. <https://doi.org/10.1371/journal.pone.0142639>
- Giampieri, F., Alvarez-Suarez, J. M., Tulipani, S., González-Paramàs, A. M., Santos-Buelga, C., & Battino, M. (2014). The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition*, 30, 9–19. <https://doi.org/10.1016/j.nut.2011.08.009>
- Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV–visible spectroscopy. In R. E. Wrolstad (Ed.), *Current Protocols in Food Analytical Chemistry*. New York: John Wiley & Sons.
- Joshi, R., Vig, A. P., & Singh, J. (2015). Vermicompost as soil supplement to enhance growth, yield and quality of plants: A review. *International Journal of Recycling of Organic Waste in Agriculture*, 4, 211–221. <https://doi.org/10.1186/2251-7715-2-16>
- Karaklajić-Stajić, Ž., Leposavić, A., Milinković, M., Paunović, S.M., & Tomić, J. (2023). Mineral composition and bioactive potential of red raspberry fruits, juice, and jam. *Zemdirb. Agric. 110*, 263–270. <https://doi.org/10.13080/z-a.2023.110.030>
- Lori, M., Symnaczik, S., Mäder, P., De Deyn, G., & Gatteringer, A. (2017). Organic farming enhances soil microbial abundance and activity—A meta-analysis and meta-regression. *Applied Soil Ecology*, 116, 147–157. <https://doi.org/10.1371/journal.pone.0180442>
- Pešaković, M., Karaklajić-Stajić, Ž., Milenković, S., & Mitrović, O. (2013). Biofertilizer affecting yield related characteristics of strawberry (*Fragaria x ananassa* Duch.) and soil micro-organisms. *Scientia Horticulturae*, 150, 238–243. <https://doi.org/10.1016/j.scienta.2012.11.016>
- Pešaković, M., Tomić, J., Cerović, R., Stampar, F., Jakopič, J., Karaklajić Stajić, Ž., Milenković, S., & Mikulic-Petkovsek, M. (2023). Evaluating effects of a new liquid vermicompost-based product on fruit quality in organic strawberries (*Fragaria × ananassa* Duch.). *Biological Agriculture & Horticulture*, 39 (7), 1–14. <https://doi.org/10.1080/01448765.2023.2217156>
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26, 1231–1237. [https://doi.org/10.1016/s0891-5849\(98\)00315-3](https://doi.org/10.1016/s0891-5849(98)00315-3)
- Richardson, A. E., Barea, J. M., McNeill, A. M., & Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil*, 321, 305–339. <https://doi.org/10.1007/s11104-009-9895-2>
- Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., De Pascale, S., & Bonini, P. (2017). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Frontiers in Plant Science*, 8, 1–15. <https://doi.org/10.1016/j.scienta.2015.09.002>
- Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152–178. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
- Tomić, J., Pešaković, M., Milivojević, J., & Karaklajić-Stajić, Ž. (2018). How to improve strawberry productivity, nutrients composition, and beneficial rhizosphere microflora by biofertilization and

- mineral fertilization? *Journal of Plant Nutrition*, 41 (16), 2009–2021. <https://doi.org/10.1080/01904167.2018.1482912>
- Tomić, J., Karaklajić-Stajić, Ž., Pešaković, M., Paunović, S. M., Rilak, B., Mikulic-Petkovsek, M., Hudina, M., Veberic, R., Kafkas, N. E., Ercişli, S., & Jakopic J. (2025). Application of vermicompost extract to improve the phytochemical composition of berry fruits. *The Turkish Journal of Agriculture and Forestry*, 49 (3), 530-542. <https://doi.org/10.55730/1300-011X.3284>
- Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Polasky, S. (2002). Agricultural sustainability and intensive production practices. *Nature*, 418, 671–677. <https://doi.org/10.1038/nature01014>
- Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., & Singh, B. K. (2020). Plant–microbiome interactions: From community assembly to plant health. *Nature Reviews Microbiology*, 18, 607–621. <https://doi.org/10.1038/s41579-020-0412-1>
- Wu, Y., Zhang, H., Li, X., & Sun, J. (2022). Plant–microbe interactions drive secondary metabolite accumulation in horticultural crops. *Frontiers in Plant Science*, 13, 932845.
- Zhang, X., Davidson, E. A., Mauzerall, D. L., Searchinger, T. D., Dumas, P., & Shen, Y. (2015). Managing nitrogen for sustainable development. *Nature*, 528, 51–59. <https://doi.org/10.1038/nature15743>

Technological characteristics of the newly introduced grapevine Souvignier gris cv. grown in the Telečka wine region

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Abstract

The study aimed to determine the technological potential of Souvignier gris, a variety introduced to Serbian viticulture in 2021, which is currently cultivated in the Telečka wine region. The research was conducted in a commercial vineyard in this region during the 2023-2025 period. Sample analysis was carried out in the laboratory of the Department of Viticulture, University of Belgrade Faculty of Agriculture. The study includes an analysis of the region's agroclimatic conditions, the mechanical composition of grapes and berries, and the quality chemical parameters of grape juice-must. In the period 2001-2020, the average annual temperature was 12.1°C, and the average vegetation temperature was 17.9°C. Annual precipitation averaged 592 mm. Mechanical composition analysis showed that the average bunch mass ranged from 156.1 to 187.9 g. The highest number of berries per bunch (185.3) was recorded in 2024. In 2023, samples had the highest average mass of 100 berries (150.0 g), the largest berry dimensions (12.5±0.7 × 11.6±1.0 mm), and the greatest skin mass of 100 berries (9.2 g). The proportion of skins in bunches ranged from 6.4% to 7.4%, while the proportion of mesocarp in bunches varied between 82.5% and 87.6%. Regarding the quality parameters of grape must, sugar content ranged from 24.0% to 26.4%, and total acidity from 8.1 to 8.7 g/L. The glycoacidometric index, expressed as the ratio of sugar to acid concentration, ranged from 2.8 to 3.1. According to these results, the examined variety meets the standards for quality white wine production in the Telečka wine region.

Keywords: Souvignier gris, grape juice, mechanical composition, sugar content, total acidity

INTRODUCTION

The introduction of new grapevine varieties to regions where they have not previously been cultivated is highly significant in viticultural practice. It broadens the existing range of cultivars and provides producers with greater flexibility in selecting varieties to grow and wine styles to produce. This fosters innovation and enhances market competitiveness. The introduced varieties may be attractive due to their agrobiological characteristics, resistance to environmental factors, or oenological potential. However, studies are required to accurately assess their performance in new vine regions (Doncieux et al., 2022).

Souvignier gris is a promising cultivar in this context. As a fungus-resistant variety, it is suitable for organic viticulture and allows for reduced protective spraying in conventional practice (Pertot et al., 2017), thereby lowering production costs. It may also contribute to the diversification of winemaking, as it is suitable for producing white wines (reminiscent of Pinot gris), as well as other styles such as orange, sparkling, and dessert wines (Schumacher et al., 2021).

The Telečka wine region forms part of the larger Vojvodina wine-growing area in northern Serbia. According to Ivanišević et al. (2016), there are approximately 115.23 ha of vineyards in this region, predominantly planted with wine varieties. Souvignier gris does not appear in the assortment list of the most recent agricultural census of Serbia, indicating that its cultivation remains local and on a small

scale. The aim of this study was to determine the technological potential of Sauvignier gris grown in the Telečka wine region, focusing on the mechanical and chemical composition of its grapes.

MATERIAL AND METHODS

Souvignier gris is a white wine variety that originated in Germany in 1983. It is also known under synonym Freiburg 392-83. It was initially thought to be a cross between Cabernet Sauvignon and Bronner; however, DNA analysis has shown that its parents are in fact Seyval blanc and Zaehring (VIVC, 2026). As one of the PIWI varieties (in German: pilzwiderstandsfähige Rebsorten – fungus-resistant grapevine cultivars), it shows resistance to powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) (Linhart et al., 2023; de Sousa Dias et al., 2025). It is an early- to medium-ripening variety (I epoch), with harvest typically in the first half of September. It is very fertile and high-yielding, requiring grape thinning in certain years.

The field experiment was conducted in a commercial vineyard in Sivac village (GPS coordinates 45.684783, 19.396984), Kula municipality, during the 2023–2025 period. The vineyard was planted in 2021, with interrow spacing of 2.4 m and a row spacing of 0.9 m. The Sauvignier gris variety was grafted onto 110 Richter rootstock. The single Guyot training system was used, with a trunk height of 90 cm. The selected experimental vines were pruned uniformly, leaving a renewal spur with 2 developed buds and a cane with 8 buds.

The technological characteristics examination of bunches was conducted in the laboratory of the Department of Viticulture, University of Belgrade Faculty of Agriculture, after harvest at full maturity. The mechanical composition of grapes and berries was determined according to the descriptive methods of Marković (2023) and Marković & Pržić (2020). The grapes were measured for weight, length, and width at full ripeness. For each of the ten selected bunches, the number of berries was counted, and the masses of berries and bunch stems per bunch were measured on an analytical balance. After measuring the mass of 100 selected berries, the berry skin and seeds were separated, dried, and measured for mass on an analytical balance. Other mechanical parameters presented in the paper were obtained by calculation.

The determined chemical parameters of grape juice-must include sugar content (°Oe and %), total acidity expressed as tartaric acid (g/L), and glycoacidometric index (GAI). GAI is expressed as the ratio of sugar to acid content. The sugar concentration was determined with an Oechsle hydrometer (SD-Duvančić, Croatia), and the values were calculated using the Dujardin-Salleron formula (Marković & Pržić, 2020 and Petrović, 2022):

$$\% \text{ sugar} = ^\circ\text{Oe} * 0.266 - 3$$

Total acidity was determined by titration with 0.1 M NaOH. Phenolphthalein was used as a colour indicator. The values were calculated according to the formula:

$$\text{Total acidity (g/L)} = \text{NaOH used} * F * 0.75$$

F - normality factor of the 0.1M NaOH solution

Statistical analysis was performed using the online statistics calculator (Statistics Kingdom, 2025). The Tukey HSD test at $p < 0.05$ was used to establish statistical differences by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Agroclimatic conditions

The climatic parameters observed in this study refer to the period 2001–2020 and to the individual years of the study: 2023, 2024, and 2025 (Republic Hydrometeorological Service of Serbia report and Digital Climate Atlas of Serbia). The climate factors examined include mean temperatures (t_s) at monthly, vegetation, and annual levels, as well as monthly and annual precipitation. The average annual (12.1°C) and vegetation temperatures (17.9°C) over the 20-year period are within the optimal range for grapevine growth (Mesa et al., 2024). Comparison of the individual study years with the longer period shows an

increase in average temperatures in recent years (especially in 2024), attributed to global warming. This may negatively affect ripening time, yield, and the composition of grape juice and wine (Torregrosa et al., 2017).

Table 1. Mean monthly (Jan-Dec), vegetation (Veg) and annual (Ann) temperatures (in °C) for the periods 2001-2020, 2023, 2024 and 2025, meteorological station Sombor

ts (°C)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Veg	Ann
2001-2020	0.7	2.4	7.2	12.7	17.4	21.1	22.8	22.4	17.2	12.0	6.9	1.8	17.9	12.1
2023	4.3	3.6	8.5	10.5	16.9	20.8	24.1	23.4	20.5	15.3	7.4	4.1	18.8	13.3
2024	2.3	9.1	10.7	14.4	18.8	23.2	26.1	26.7	18.2	12.5	4.5	2.4	20.0	14.1
2025	3.1	2.1	9.4	14.0	15.8	23.1	23.6	23.1	20.7	12.2	7.2	3.8	18.9	13.2

Mean monthly precipitation during the observed periods is shown in Figure 1. The average annual rainfall over the 20-year period was 592 mm. The highest total precipitation occurred in 2023 (679 mm), followed by 610 mm in 2024, while the lowest was recorded in 2025 (480 mm). Lower precipitation during the ripening period (July and August in Figure 1) allows for adequate grape maturation, as high rainfall leads to sugar dilution and an increased risk of diseases (Santos et al., 2020; Licurici et al., 2025). Overall, the region is suitable for grapevine cultivation.

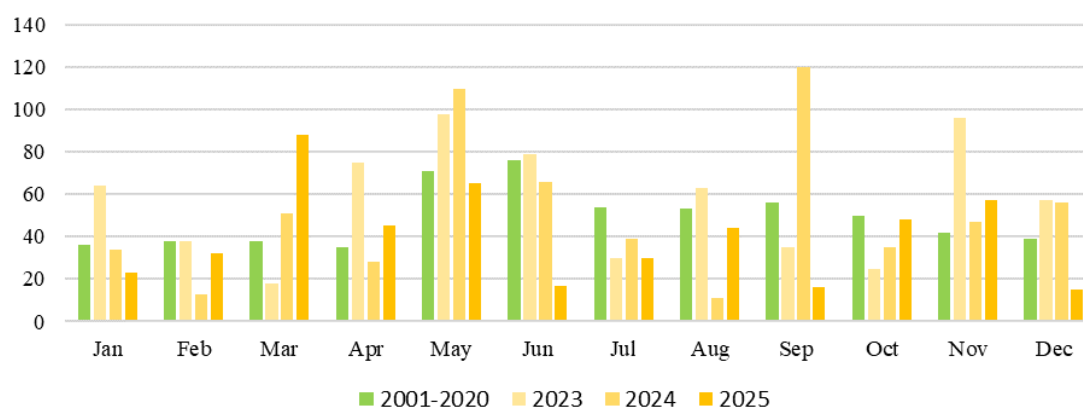


Figure 1. Mean precipitation (mm) for the periods 2001-2020, 2023, 2024 and 2025, meteorological station Sombor

Mechanical composition of bunches and berries

The mechanical composition of grapes is characterised by the mass ratio of the morphological parts of the bunch. It is an important scientific and practical indicator for estimating oenological potential and depends on grapevine variety, environmental conditions, and the applied ampelotechnical operations (Makuev et al., 2022; Pržić et al., 2025).

No statistically significant differences were found in the average bunch masses for 2023 (156.1 ± 50.3 g), 2024 (178.4 ± 35.6 g), and 2025 (187.9 ± 25.1 g). Similarly, there were no significant differences in the average mass of berries per bunch (Table 2). Tukey HSD indicated statistically significant differences in the average number of berries per bunch, with 2024 (185.2 ± 50.1) and 2025 (174.0 ± 22.4) both significantly higher than 2023 (108.8 ± 36.5). The same trend was observed for the average mass of berries per bunch, with the lowest value in 2023 (150.4 ± 48.8 g). However, the highest average mass of 100 berries (150.0 g), berry dimensions ($12.5 \pm 0.7 \times 11.6 \pm 1.0$ mm), and skin mass of 100 berries (9.2 g) were recorded in 2023.

Table 2. Souvignier gris bunches mechanical composition

Parameter	Year		
	2023	2024	2025
Bunch mass (g)	156.1 ± 50.3 ^{ns}	178.4 ± 35.6 ^{ns}	187.9 ± 25.1 ^{ns}
Bunch length (cm)	12.9 ± 1.8 b	16.2 ± 1.4 a	14.6 ± 0.9 a
Bunch width (cm)	7.1 ± 1.3 b	10.1 ± 1.8 a	8.4 ± 1.4 b
Number of berries in the bunch	108.8 ± 36.5 b	185.2 ± 50.1 a	174.0 ± 22.4 a
Mass of berries in the bunch (g)	150.4 ± 48.8 ^{ns}	170.9 ± 35.5 ^{ns}	182.3 ± 24.4 ^{ns}
Mass of 100 berries (g)	150.0	115.0	113.0
Skin mass of 100 berries (g)	9.2	6.0	7.9
Seeds mass of 100 berries (g)	3.7	6.0	5.7
Seeds per 100 berries	129.0	233.0	180.0
Mass of 100 seeds (g)	2.9	3.0	3.2
Mass of bunch stem (g)	5.3 ± 2.6 ^{ns}	7.2 ± 2.0 ^{ns}	6.3 ± 1.3 ^{ns}
Berry dimensions	Length (mm)	12.5 ± 0.7 ^{ns}	11.2 ± 1.5 ^{ns}
	Width (mm)	11.6 ± 0.1 ^{ns}	11.4 ± 0.9 ^{ns}

Letters indicate significant differences - Tukey's test ($p < 0.05$); ^{ns} - not significant.

Samples in 2023 had the highest average mass per berry (1.50 g) and therefore the lowest berry indicator (69.70). The highest number of seeds per bunch was recorded in 2024 (431.52), resulting from those samples having the most berries per bunch, as well as the highest number of seeds per berry (2.33).

Table 3. Souvignier gris bunch and berry mechanical composition

Parameter	Year		
	2023	2024	2025
Average mass of one berry (g)	1.50	1.15	1.13
Average skin mass of one berry (g)	0.09	0.06	0.08
Average seed mass of one berry (g)	0.04	0.06	0.06
Number of seeds in one berry	1.29	2.33	1.80
Mass of 100 seeds (g)	2.87	2.57	3.17
Mass of one seed (g)	0.03	0.03	0.03
Mesocarp mass in 100 berries (g)	137.06	103.00	99.33
Berry mass in bunch (g)	150.80	160.80	181.60
Skin mass in bunch (g)	10.05	11.11	13.87
Seed mass in bunch (g)	4.03	11.11	9.92
Mesocarp mass in bunch (g)	136.72	138.58	157.81
Number of seeds in a bunch	140.35	431.52	313.20
% of skins in a berry	6.16	5.22	7.05
% of seeds in a berry	2.47	5.22	5.04
% of mesocarp in a berry	91.37	89.56	87.90
Berry indicator	69.70	110.24	92.60
Berry weight indicator	14.83	17.17	12.46
Bunch skeleton	9.83	10.90	10.73
Hard rest of a bunch	12.41	17.51	16.01
Bunch weight indicator	28.45	22.33	28.82
Bunch structure indicator	7.05	4.71	5.24

According to the bunch structure analysis (Figure 2), the proportion of skins in a bunch ranged from 6.4% to 7.4%. A larger proportion of skins in grapes contributes to increased varietal aroma and phenolic content in wines, especially if skin contact is included during fermentation (e.g., orange wines) (Sokolowsky et al., 2015; Sancho-Galán et al., 2021). A higher percentage of mesocarp, as observed in 2023 (87.6%), results in a greater amount of grape must that can be extracted from the grapes.

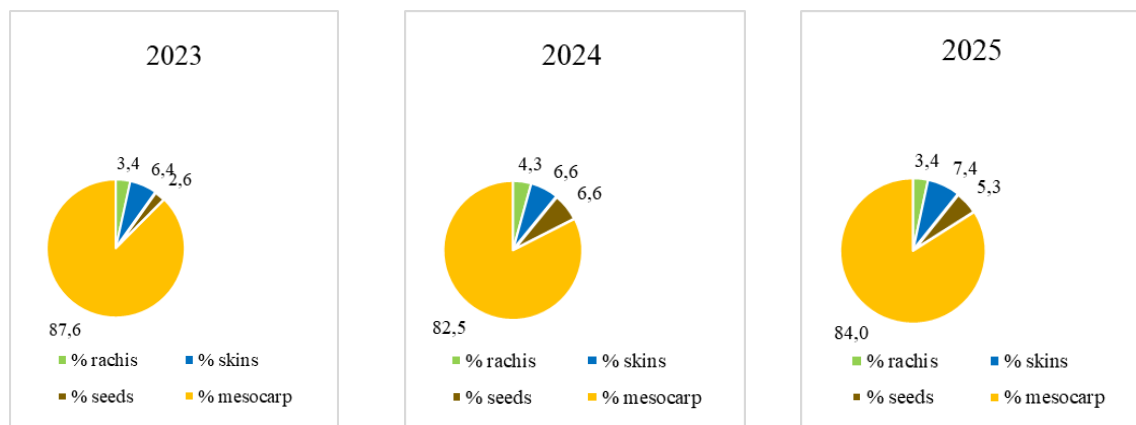


Figure 2. Souvignier gris bunch structure indicators (%)

Chemical composition of grape juice-must

The primary quality indicators of grape juice-must, in terms of chemical composition, are sugar content and total acidity (Table 4). Grapes were harvested at full maturity, that is, when sugar accumulation had ceased. The harvest dates were 6 September 2023, 17 August 2024, and 19 August 2025. Sugar concentration ranged from 101.4 to 110.6 °Oe, equivalent to 24.0 to 26.4%. According to Winkler (as cited in Žunić & Garić, 2017), the typical carbohydrate range in grape must is 15 to 25%. An optimal sugar level is important, as it directly influences the amount of alcohol produced during fermentation. Alcohol is necessary for the stability and proper ageing of wine, as well as its sensory perception. However, in years when grapes accumulate high sugar levels, as observed in 2024 (26.4%), the alcohol content of wine can be excessively high. This can potentially lead to fermentation issues and compromise the wine's taste by affecting its aroma, astringency, and acidity (Martínez-Pérez, 2020). Total acidity varied between 8.1 and 8.7 g/L. For comparison, Humaj et al. (2023) report lower acidity in Souvignier gris grape must (7.6 g/L) in their study in Poland. Blackford et al. (2025) report that Souvignier gris had the highest titratable acidity of all examined fungal-resistant varieties in their study. Titratable acidity for the production of well-balanced wines ranges from 6.5 to 8.5 g/L (Keller, 2020). Sufficient acid content is necessary as it enhances wine stability and organoleptic qualities (Plantevin et al., 2024), especially in white wines (Ribéreau-Gayon et al., 2021). The established tendency of Souvignier gris to accumulate higher acid levels is particularly important today in light of global warming, as rising temperatures lead to lower acidity in grapes (Payan et al., 2023). The glycoacidometric index in samples varied between 2.8 and 3.1. Overall, the chemical composition analysis has shown that Souvignier gris meets the requirements for quality wine production.

Table 4. Souvignier gris grape juice-must chemical composition

Year	Sugar content		Total acidity (g/L)	Glycoacidometric index
	°Oe	%		
2023	106.0	25.2	8.1	3.1
2024	110.6	26.4	8.4	3.1
2025	101.4	24.0	8.7	2.8

CONCLUSION

The review of agroclimatic conditions from 2001 to 2020 has shown that the Telečka wine region is suitable for grapevine growth and cultivation. Temperature sums, rainfall amounts, and their distribution throughout the year are satisfactory. Certain years with increased temperature extremes during the maturation period, such as 2024, can lead to accelerated ripening and earlier harvest dates. Regarding mechanical composition, no statistically significant differences were found in the average bunch mass during the experiment. In 2023, samples had a significantly lower average number of berries per bunch compared to 2024 and 2025, with the same trend observed for the average mass of berries per bunch. Grapes in 2023 were characterised by the largest average mass of a single berry and the lowest berry indicator. The highest number of seeds per berry and per bunch was observed in 2024. Chemical composition analysis has shown that the grapes accumulate high sugar levels and maintain good acid content in all years of the research. The variety meets the standard for quality white wine production. Based on these results, Sauvignier gris can be recommended for further planting in the Telečka wine region and for enriching the Serbian grapevine varietal assortment.

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REFERENCES

- Blackford, M., Guggenbühl-Gasser, B., Inderbitzin, J., Wiederkehr, M., Fuchsmann, P., Bieri, S., & Mackie-Haas, K. (2025). Consumer preferences in wine: traditional vs fungus-resistant varieties in Switzerland. *OENO One*, 59(3). <https://doi.org/10.20870/oenone.2025.59.3.9239>
- de Sousa Dias, A. L., Guittin-Leignadier, C., Roy, A., Sachot, S., Maçna, F., Flores, D., Meudec, E., Boulet, J., Sommerer, N., & Mouret, J. R. (2025). Oenological Performances of New White Grape Varieties. *Beverages*, 11(3), 90. <https://doi.org/10.3390/beverages11030090>
- Digital Climate Atlas of Serbia: <https://atlas-klima.eko.gov.rs/lat/map?dataType=obs&visualization=pro&area=regions> (February 2026).
- Doncieux, A., Yobrégat, O., Prudham, S., Caillon, S., & Renard, D. (2022). Agrobiodiversity dynamics in a French wine-growing region. *OENO One*, 56(4), 183-199. <https://doi.org/10.20870/oenone.2022.56.4.5557>
- Humaj, J., Kostelnikova, K., Sochor, J., Kumsta, M., & Baron, M. (2023). Study of polyphenolic compounds in wines and different parts of the grapevine. *Folia Horticulturae*, 35, 297-306. <https://doi.org/10.2478/fhort-2023-0022>
- Ivanišević, D., Jakšić, D., & Korać, N. (2016). Viticulture Atlas. Statistical Office of the Republic of Serbia. <https://www.stat.gov.rs/en-us/oblasti/poljoprivreda-sumarstvo-i-ribarstvo/popis-poljoprivrede/>
- Keller, M. (2020). *The science of grapevines*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-816365-8.00006-3>
- Licurici, M., Vlăduț, A. Ș., & Burada, C. D. (2025). A Study of Observed Climate Change Effects on Grapevine Suitability in Oltenia (Romania). *Horticulturae*, 11(6), 591. <https://doi.org/10.3390/horticulturae11060591>
- Linhart, L., Moretti, B., Herrera, J. C., & Forneck, A. (2023). Maximum stomatal conductance rather than stomatal sensitivity to drought differentiates the PIWI grapevine cultivar Sauvignier gris from Muscaris and Donauriesling. *OENO One*, 57(2), 129-138. <https://doi.org/10.20870/oenone.2023.57.2.7307>
- Makuev, G. A., Isrigova, T. A., Mukailov, M. D., Salmanov, M. M., & Magomedov, M. G. (2022, February). Technological assessment of native grapes varieties for winemaking in the conditions of Southern Dagestan. In *IOP Conference Series: Earth and Environmental Science* (Vol. 979, No. 1, p. 012018). IOP Publishing. <https://doi.org/10.1088/1755-1315/979/1/012018>

- Marković, N. (2023). *Tehnologija gajenja vinove loze* [Technology of Viticulture]. Zadužbina svetog Manastira Hilandara; Poljoprivredni fakultet Univerziteta u Beogradu.
- Marković, N., & Pržić, Z. (2020). *Tehnologija gajenja vinove loze* [Technology of Viticulture - Practicum]. Zadužbina svetog Manastira Hilandara; Poljoprivredni fakultet Univerziteta u Beogradu.
- Martínez-Pérez, M. P., Bautista-Ortín, A. B., Pérez-Porras, P., Jurado, R., & Gómez-Plaza, E. (2020). A new approach to the reduction of alcohol content in red wines: The use of high-power ultrasounds. *Foods*, 9(6), 726. <https://doi.org/10.3390/foods9060726>
- Mesa, I. F., Sánchez-Costa, E., Aranda, X., Altava-Ortiz, V., Barrera-Escoda, A., Prohom, M., Poyatos, R., Sánchez-Ortiz, A., Savé, R., & de Herralde, F. (2024). Modelling future climate change impacts on grapevine water requirements and growing cycle in three wine PDOs of NE Spain. *OENO One*, 58(4). <https://doi.org/10.20870/oeno-one.2024.58.4.7789>
- Payan, C., Gancel, A. L., Jourdes, M., Christmann, M., & Teissedre, P. L. (2023). Wine acidification methods: A review. *OENO One*, 57(3), 113-126. <https://doi.org/10.20870/oeno-one.2023.57.3.7476>
- Pertot, I., Caffi, T., Mugnai, L., Hoffmann, C., Grando, M. S., Gary, C., Lafond, D., Duso, C., Theiry, D., Mazzoni, V., & Anfora, G. (2017). A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. *Crop Protection*, 97: 70-84. <https://doi.org/10.1016/j.cropro.2016.11.025>
- Petrović, A. (2022). *Praktikum za tehnologiju vina* [Practicum for Wine technology]. Poljoprivredni fakultet Univerziteta u Beogradu.
- Plantevin, M., Merpault, Y., Lecourt, J., Destrac-Irvine, A., Dijsktra, L., & van Leeuwen, C. (2024). Characterization of varietal effects on the acidity and pH of grape berries for selection of varieties better adapted to climate change. *Frontiers in Plant Science*, 15, 1439114. <https://doi.org/10.3389/fpls.2024.1439114>
- Pržić, Z., Marković, N., & Živadinović, Đ. (2025). Technological and oenological characteristics of the Ferdinand de Lesseps and Muskat ruža wine grape varieties. *Agroзнanje*, 26(1), 57-73. <https://doi.org/10.7251/AGREN2501057P>
- Republic Hydrometeorological Service of Serbia: <https://www.hidmet.gov.rs/> (February 2026).
- Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2021). *Handbook of Enology, Volume 2: The chemistry of wine stabilization and treatments*. John Wiley & Sons.
- Sancho-Galán, P., Amores-Arrocha, A., Jiménez-Cantizano, A., & Palacios, V. (2021). Influence of the presence of grape skins during white wine alcoholic fermentation. *Agronomy*, 11(3), 452. <https://doi.org/10.3390/agronomy11030452>
- Santos, J. A., Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Dinis, L. T., Correia, C., Moriondo, M., Leolini, L., Dibari, C., Costafreda-Aumedes, S., Kartschall, T., Menz, C., Molitor, D., Junk, J., Beyer, M., & Schultz, H. R. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. *Applied Sciences*, 10(9), 3092. <https://doi.org/10.3390/app10093092>
- Schumacher, P., Mackie-Haas, K., & Wins, T. (2021). Sauvignier gris: Eine Piwi-Sorte mit grossem Potenzial. *Obst+Wein*, 8: 16-18.
- Sokolowsky, M., Rosenberger, A., & Fischer, U. (2015). Sensory impact of skin contact on white wines characterized by descriptive analysis, time-intensity analysis and temporal dominance of sensations analysis. *Food Quality and Preference*, 39, 285-297. <https://doi.org/10.1016/j.foodqual.2014.07.002>
- Torregrosa, L., Bigard, A., Doligez, A., Lecourieux, D., Rienth, M., Luchaire, N., Pieri, P., Chatbanyong, R., Shahood, R., Farnos, M., Roux, C., Adivize, A., Pillet, J., Sire, Y., Zumstein, E., Veyret, M., Le Cunff, L., Lecourieux, F., Saurin, N., ... & Romieu, C. (2017). Developmental, molecular and genetic studies on grapevine response to temperature open breeding strategies for adaptation to warming. *Oeno One*, 51(2), 155-165. <https://doi.org/10.20870/oeno-one.2017.51.2.1587>
- Vitis International Variety Catalogue VIVC. (2026). *Souvignier gris*. <https://www.vivc.de/index.php?r=passport%2Fview&id=22629> (February 2026).
- Žunić, D., & Garić, M. (2017). *Posebno vinogradarstvo* [Special Viticulture]. Poljoprivredni fakultet Univerziteta u Beogradu; Poljoprivredni fakultet Univerzieta u Prištini.

Morphological characteristics of one-year-old walnut (*Juglans regia* L.) shoots

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Abstract

The objective of this research was to determine the influence of genotype (three spontaneous seedlings: L16, L6 and L45), environmental factors over two years (2020–2021), shoot length (<6 cm, 8–18 cm, and >20 cm), and shoot type (shoots developed from vegetative buds versus those from mixed buds) on the morphological characteristics of one-year-old shoots (diameter, node number, and length-to-diameter ratio). Furthermore, the study examined the representation of different bud categories (vegetative, dormant catkins, and mixed buds) on the three terminal nodes and across the entire one-year-old walnut shoots, as well as the representation of different shoot types within the overall structure of the one-year-old wood. The results showed that, in most cases, genotype, shoot length, and shoot type exerted a significant influence on the investigated parameters, whereas the effect of the year was not significant.

Keywords: genotype, vegetative shoots, fruiting shoots, mixed buds, dormant catkins

INTRODUCTION

The walnut is a monoecious species with male and female flowers in separate buds. Three bud types are distinguished on annual shoots: vegetative, mixed, and catkin primordia (Germain *et al.*, 1975). The terminal bud occupies the apex, while lateral buds are situated in leaf axils, either individually or in a serial arrangement (Polito, 1998).

Vegetative buds produce shoots that lignify into annual shoots by the season's end, either retaining their vegetative character or forming generative organs (Lučić *et al.*, 1996). Catkins, the precursors of male inflorescences, resemble small coniferous cones during dormancy and develop in leaf axils during the year preceding flowering (Milatović *et al.*, 2020). Mixed buds, which form fructification shoots with apical female flowers, are dome-shaped and larger than spherical lateral vegetative buds (Mičić *et al.*, 1987). After fruiting, these shoots terminate apical growth, leaving fruit scars, while subsequent development continues from lateral points, creating complex branched fruiting wood (Mičić, 1992). They form fructification shoots that bear female flowers at the apex. After ripening, these become annual shoots with terminated apical growth, where fruit or flower scars are found at the terminal position instead of a bud, and proleptic (syllaptic) shoots form from 1-2 subterminal buds. The further development of shoots derived from mixed buds continues from lateral growth points, forming a complex (branched) fruiting wood as a category of old fruiting wood (Mičić, 1992).

Based on bud arrangement, annual shoots are classified as vegetative, male, female fruiting, or combined (Mičić *et al.*, 1987). Bearing habits are categorised as terminal, intermediate (10–40% lateral mixed buds), or lateral (>40% lateral mixed buds) (Dzhuvinov & Gandev, 2014). Since node numbers and generative proportions depend on genotype, age, and environmental factors (Solar & Štampar, 2003;

Lučić *et al.*, 1996), maintaining the correct ratio of winter buds is vital for balancing growth and productivity (Mičić *et al.*, 1987).

The objective of this research was to determine the influence of genotype, environmental factors (year), shoot length, and shoot origin on the morphometric characteristics and bud structure of walnut annual shoots, as well as on the representation of different shoot types (vegetative and fruiting) within the overall structure of one-year-old wood.

MATERIALS AND METHODS

Plant material and growing conditions

The research was conducted on three walnut genotypes from a spontaneous population in the village of Lazac (near Kraljevo, central Serbia): L16 (protandrous, early leafing); L6 (protogynous, mid-early leafing); and L45 (protogynous, late leafing).

The trees were in full bearing age, grown without specific training systems (free-growth form). Standard agrotechnical measures were applied (mowing, weed control using glyphosate-based herbicides, and occasional fertilisation with manure and mineral fertilisers). No irrigation was applied.

During the three-month period from May to July, which is critical for walnut shoot growth, precipitation amounted to 292 mm in 2020 and 239.3 mm in 2021 (Table 1).

Table 1. Mean temperatures and precipitation in Kraljevo (43°42' N, 20°42' E, 215 m) in the period 2020–2022 (Republic Hydrometeorological Service of Serbia, 2021).

Month	2020		2021	
	Mean monthly air temperatures (°C)	Monthly precipitation totals (mm)	Mean monthly air temperatures (°C)	Monthly precipitation totals (mm)
January	0.9	22.0	1.8	119.4
February	5.5	33.5	3.7	28.1
March	7.1	57.0	5.3	25.2
April	11.5	16.8	9.1	46.3
May	15.2	79.9	15.4	114.3
June	19.1	167.4	21.3	43.9
July	22.0	44.7	23.6	81.1
August	22.9	80.0	21.4	50.0
September	18.3	33.6	16.3	21.2
October	13.0	135.1	10.2	67.6
November	5.4	11.2	7.7	26.1
December	3.8	62.6	3.1	97.0
Annual average	12.1	743.8	11.6	720.2

Morphological characteristics of one-year-old shoots

The experiment was set up as a four-factor design (genotype, year, shoot length, and shoot type), in which the main effects of the factors, as well as the interactions between the factors, were tested within the statistical analysis.

The plant material comprised selected walnut genotypes identified in a spontaneous population and considered as specific model genotypes for comparative evaluation. As each genotype was represented by a

single, unique tree, replicate trees per genotype were not available. During dormancy (2020 and 2021), 60 shoots per genotype were sampled from different parts of the canopy and separated into two groups of 30 based on their origin: (1) shoots arising from vegetative buds (VBS) and (2) shoots arising from mixed buds (MBS) that had borne mature fruit. Within each group, shoots were further allocated to three predefined length classes (<6 cm, 8–18 cm, and >20 cm), with 10 shoots per class. Accordingly, the sampled shoots represented subsamples within a tree rather than true biological replicates at the genotype level.

Shoot length was measured from the base to the tip of the terminal bud (for VBS) or to the top of the fruit scar (for MBS), and these measurements were used solely for classification into length classes and for the calculation of length-based indices. Therefore, shoot length was treated as a grouping factor rather than as an independent morphological response variable. Samples were stored in polyethylene bags in a refrigerator until analysis.

The analysis of morphological parameters included: (1) shoot length from the base to the tip of the terminal bud (for shoots from vegetative buds) or to the top of the fruit scar (for shoots from mixed buds); (2) shoot thickness was measured just above the basal scale scars using a caliper and recorded in millimeters (mm) with an accuracy of 0.1 mm; (3) elongation index (length-to-thickness ratio); (4) number of nodes per shoot; (5) analysis of the type and representation of catkins, vegetative, and mixed buds on the three terminal nodes; and (6) average number of catkins and buds per node (calculated as the ratio of the total number of organs to the number of nodes).

Bud types were identified using a binocular microscope (10-60× magnification). Buds with at least one female flower primordium were classified as mixed. Based on bud composition, shoots were categorised into four groups: vegetative, staminate (male), pistillate (female) fruiting, and combined fruiting shoots.

Statistical data analysis

Results are presented as mean value $\pm$ standard deviation (SD). Data were processed using analysis of variance (ANOVA). Significance levels are denoted as: ns: not significant; *: significant ($p < 0.05$); **: highly significant ($p < 0.01$); ***: very highly significant ($p < 0.001$). [U7.1] The significance of differences between mean values was tested using Tukey's test at a significance level of $P < 0.05$. Means in columns followed by the same letter did not differ significantly. The relationship between parameters was examined using regression and correlation analysis, with the strength of the association determined by the Pearson coefficient (r) at a significance level of $P \leq 0.05$ and $P \leq 0.01$. A formal correlation matrix was not included in the Results section due to the high number of variables with low and non-significant coefficients. Instead, only the most relevant and significant correlations are highlighted and discussed within the text. Correlation strength was interpreted according to Stanković et al. (2002): weak ($|r| \leq 0.50$), moderate ($0.50 < |r| \leq 0.75$), strong ($0.75 < |r| \leq 0.90$), and very strong ($|r| > 0.90$). Data processing was performed using SPSS software (version 31).

RESULTS

Morphological characteristics of one-year-old shoots

Shoot length and shoot type had a very highly significant effect on shoot thickness; genotype had a highly significant effect, while the year of study had a significant influence (Table 2). The highest average thickness was recorded in genotype L45 (8.75 mm), while the lowest was found in genotype L16 (8.13 mm). In 2020, shoots had an average thickness of 8.60 mm, whereas in 2021, the thickness was lower (8.25 mm). Shoots shorter than 6 cm had the smallest thickness (6.83 mm), while shoots longer than 20 cm had the largest (10.02 mm). The average thickness of VBS was 7.75 mm, which was 1.35 mm less than in MBS. Shoot thickness showed a moderately strong positive correlation with the number of nodes per shoot ($r = 0.77^{**}$), shoot length ($r = 0.61^{**}$), the number of mixed buds per shoot ($r = 0.57^{**}$), and the number of vegetative buds per shoot ($r = 0.52^{**}$).

Shoot length and shoot type had a very highly significant effect on the number of nodes per shoot, genotype had a highly significant effect, while the influence of the year was not significant. The highest average number of nodes per shoot was determined in genotypes L6 (9.07) and L45 (8.96), and the lowest in L16 (8.46). The average number of nodes on shoots shorter than 6 cm was 7.34, while it was

highest on shoots over 20 cm in length (10.17). Longer shoots had a significantly higher number of nodes than shorter ones. VBS had, on average, 0.87 fewer nodes than MBS. The number of nodes per shoot was in a moderately strong positive correlation with the number of vegetative buds per shoot ($r = 0.66^{**}$), shoot length ($r = 0.57^{**}$), and the number of vegetative buds per node ($r = 0.56^{**}$). Conversely, it showed a moderately strong negative correlation with the number of catkins per node ($r = -0.51^{**}$) and a weak negative correlation with the number of catkins per shoot ($r = -0.21^{**}$). The interaction between shoot length $\times$ shoot type significantly influenced the number of nodes per shoot, reflected in the fact that for shoots longer than 20 cm, the difference in node number was less pronounced.

The year of study had no statistically significant effect on shoot elongation index, whereas the effect of genotype was significant, and the effects of shoot length and shoot type were very highly significant (Table 2). The highest elongation index was observed in genotype L16 (13.55) and the lowest in L45 (12.64). For shoots longer than 20 cm, the elongation index was more than twice as high as for those in the <6 cm and 8–18 cm ranges. The lowest elongation index was recorded in shoots shorter than 6 cm (9.00). In VBS, the elongation index was 14.07, which was 1.91 higher than in MBS.

Table 2. Morphological characteristics of shoots

Factor	Factor levels	Shoot base diameter (mm)	Number of nodes per shoot	Shoot elongation index
Genotype (G)	L6	8.37 $\pm$ 1.91 ab	9.07 $\pm$ 1.89 b	13.17 $\pm$ 5.93 ab
	L16	8.13 $\pm$ 1.92 a	8.46 $\pm$ 1.80 a	13.55 $\pm$ 6.03 b
	L45	8.75 $\pm$ 2.00 b	8.96 $\pm$ 1.87 b	12.64 $\pm$ 5.55 a
Year (Y)	2020	8.60 $\pm$ 2.06 b	8.90 $\pm$ 1.92 a	12.88 $\pm$ 5.75 a
	2021	8.25 $\pm$ 1.85 a	8.64 $\pm$ 1.80 a	13.35 $\pm$ 5.89 a
Shoot length (L)	< 6 cm	6.83 $\pm$ 1.13 a	7.34 $\pm$ 1.33 a	9.00 $\pm$ 1.38 a
	8–18 cm	8.43 $\pm$ 1.50 b	8.79 $\pm$ 1.60 b	9.76 $\pm$ 1.57 b
	>20 cm	10.02 $\pm$ 1.73 c	10.17 $\pm$ 1.45 c	20.58 $\pm$ 3.63 c
Shoot type (T)	VBS	7.75 $\pm$ 1.61 a	8.33 $\pm$ 1.96 a	14.07 $\pm$ 5.97 b
	MBS	9.10 $\pm$ 2.06 b	9.20 $\pm$ 1.66 b	12.16 $\pm$ 5.51 a
ANOVA				
G		**	**	*
Y		*	ns	ns
L		***	***	***
T		***	***	***
L $\times$ T		ns	*	ns

Factor interactions that did not exert a statistically significant effect were omitted from the table.

Number and distribution of vegetative buds on shoots

Shoot length is the only highly significant factor affecting the number of vegetative buds on the first node (Table 3). Shoots shorter than 6 cm had a significantly higher number of buds (0.33) compared to longer shoots. No statistically significant differences were observed between shoots 8–18 cm and >20 cm.

Shoot length and type significantly influenced the number of vegetative buds on the second node, whereas genotype and year did not. Shoots >20 cm had a very highly significantly higher count (0.72) than those 8–18 cm (0.36) or <6 cm (0.29). VBS had 0.52 vegetative buds compared to 0.40 in MBS. Both genotype $\times$ shoot type and shoot length $\times$ shoot type interactions were significant.

Shoot length and genotype exerted significant effects on the number of vegetative buds on the third node, while year and shoot type did not. Genotype L45 recorded more buds (0.61) than L6 and L16 (both 0.40). The count ranged from 0.23 on shoots <6 cm to 0.79 on those >20 cm, with a very highly

significant shoot length $\times$ shoot type interaction.

The vegetative bud count per shoot was influenced very highly significantly by genotype and shoot length, but not by year or type. L45 had the highest (4.76) and L16 the lowest (2.56) count (Table 3), with no significant difference between L6 and L16. Shoots >20 cm averaged 5.69 buds, significantly higher than shorter categories (3.01 and 2.06), between which differences were non-significant.

Moderately strong positive correlations were found between vegetative buds per shoot and node count ($r = 0.629^{**}$), shoot thickness ($r = 0.500^{**}$), and third-node buds ($r = 0.638^{**}$). Conversely, moderately strong negative correlations existed with catkins per shoot ($r = -0.567^{**}$) and per node ($r = -0.684^{**}$). Both genotype $\times$ shoot type and shoot length $\times$ shoot type interactions were highly significant.

Genotype and shoot length were very highly significant factors for the number of vegetative buds per node. L45 had the highest (0.47) and L16 the lowest (0.28) values (Table 3). Counts were highest on shoots >20 cm (0.54) and lowest on those <6 cm (0.26), with non-significant differences between shorter categories. Both primary interactions (genotype $\times$ type and length $\times$ type) were highly significant.

Table 3. Number and distribution of vegetative buds on shoots

Factor	Factor levels	Number of vegetative buds				
		On the 1 st node	On the 2 nd node	On the 3 rd node	Per shoot	Per node
Genotype (G)	L6	0.08 $\pm$ 0.25 a	0.30 $\pm$ 0.59 a	0.40 $\pm$ 0.58 a	3.28 $\pm$ 3.44 a	0.33 $\pm$ 0.30 a
	L16	0.16 $\pm$ 0.35 a	0.40 $\pm$ 0.61 a	0.40 $\pm$ 0.57 a	2.56 $\pm$ 3.00 a	0.28 $\pm$ 0.28 a
	L45	0.22 $\pm$ 0.70 a	0.54 $\pm$ 0.68 a	0.61 $\pm$ 0.70 b	4.76 $\pm$ 4.95 b	0.47 $\pm$ 0.43 b
Year (Y)	2020	0.13 $\pm$ 0.50 a	0.41 $\pm$ 0.65 a	0.44 $\pm$ 0.62 a	3.65 $\pm$ 4.28 a	0.36 $\pm$ 0.37 a
	2021	0.20 $\pm$ 0.53 a	0.50 $\pm$ 0.62 a	0.53 $\pm$ 0.65 a	3.52 $\pm$ 3.88 a	0.37 $\pm$ 0.35 a
Shoot length (L)	< 6 cm	0.33 $\pm$ 0.46 a	0.29 $\pm$ 0.44 a	0.23 $\pm$ 0.46 a	2.06 $\pm$ 2.53 a	0.26 $\pm$ 0.27 a
	8–18 cm	0.13 $\pm$ 0.71 b	0.36 $\pm$ 0.59 a	0.43 $\pm$ 0.60 b	3.01 $\pm$ 4.08 a	0.30 $\pm$ 0.36 a
	>20 cm	0.04 $\pm$ 0.18 b	0.72 $\pm$ 0.76 b	0.79 $\pm$ 0.70 c	5.69 $\pm$ 4.48 b	0.54 $\pm$ 0.39 b
Shoot type (T)	VBS	0.11 $\pm$ 0.29 a	0.52 $\pm$ 0.69 b	0.40 $\pm$ 0.63 a	3.16 $\pm$ 4.21 a	0.32 $\pm$ 0.37 a
	MBS	0.20 $\pm$ 0.66 a	0.40 $\pm$ 0.57 a	0.56 $\pm$ 0.63 a	4.01 $\pm$ 3.91 a	0.41 $\pm$ 0.35 a
ANOVA						
G		ns	ns	*	***	***
Y		ns	ns	ns	ns	ns
L		**	***	***	***	***
T		ns	*	ns	ns	ns
G $\times$ T		ns	*	ns	***	***
L $\times$ T		ns	***	***	**	**

Factor interactions that did not exert a statistically significant effect were omitted from the table.

Number and distribution of mixed buds on shoots

Shoot length had a very highly significant effect on the number of mixed buds on the first node, while shoot type had a significant influence (Table 4). On the shortest shoots, the number of mixed buds on the first node was lower than on shoots longer than 8 cm. Regarding this parameter, there were no differences between shoots 8–18 cm long and those over 20 cm. VBS had an average of 0.91 mixed buds on the first node, while MBS had 0.84.

Shoot length and shoot type had a very highly significant effect on the number of mixed buds on the second node; genotype had a significant influence, while the effect of the year was not significant (Table 4). The lowest number of mixed buds on the second node was recorded in genotype L16 (0.33), and the highest in genotype L6 (0.48). Increasing shoot length positively affected the number of mixed buds on the second

node. Shoots longer than 20 cm had an average of 0.47 mixed buds on the second node, while their presence was lowest in shoots shorter than 6 cm (0.24). VBS had an average of 0.07 mixed buds on the second node, while in MBS, this value was ten times higher (0.7). The interaction between shoot length $\times$ shoot type exerted a significant influence on the number and distribution of mixed buds on shoots.

Shoot type had a very highly significant effect on the number of mixed buds on the third node; genotype and shoot length had a significant influence, while the effect of the year was not significant (Table 4). In genotype L6, the average number of mixed buds on the third node was the highest (0.35), while for the other two genotypes, the difference was not significant (0.20–0.22). On shoots over 20 cm, the number of mixed buds on the third node was the highest (0.34), and it was lowest in shoots smaller than 6 cm (0.15). The average number of mixed buds on the third node for VBS was 0.04, and for MBS it was 0.43.

Shoot length and shoot type had a very highly significant effect on the number of mixed buds per shoot; genotype had a significant influence, while the effect of the year was not significant (Table 4). The lowest number of mixed buds was determined in shoots of genotype L16 (1.48), and the highest in shoots of genotype L6 (1.95). Longer shoots had a higher number of mixed buds. Shoots up to 6 cm had an average of 1.12 mixed buds, shoots 8–18 cm long had 1.72 buds, while shoots longer than 20 cm contained an average of 1.99 mixed buds. VBS had an average of 1.06 mixed buds, while MBS had 2.15.

The interaction between shoot length and shoot type had a significant influence on the number of mixed buds per shoot. The number of mixed buds per shoot was in a moderately strong positive correlation with the number of nodes per shoot ($r = 0.538^{**}$) and shoot thickness ($r = 0.596^{**}$).

The year of study had no significant effect on the number of mixed buds per node; genotype exerted a significant influence, the effect of shoot length was highly significant, while the effect of shoot type was very highly significant. The fewest mixed buds per node were recorded in genotype L6 (0.21), while in the other two genotypes, this value was 0.17. Shoots shorter than 6 cm had the fewest mixed buds per node (0.15), while in both categories of longer shoots, this parameter was 0.19. VBS had 0.13 mixed buds per node, and MBS had 0.23. The interaction between shoot length and shoot type had a significant influence on the number of mixed buds per node.

Table 4. Number and distribution of mixed buds on shoots

Factor	Factor levels	Number of mixed buds				
		On the 1 st node	On the 2 nd node	On the 3 rd node	Per shoot	Per node
Genotype (G)	L6	0.95 $\pm$ 0.22 a	0.48 $\pm$ 0.50 b	0.35 $\pm$ 0.47 b	1.95 $\pm$ 1.08 b	0.21 $\pm$ 0.10 b
	L16	0.85 $\pm$ 0.36 a	0.33 $\pm$ 0.47 a	0.22 $\pm$ 0.40 a	1.48 $\pm$ 1.08 a	0.17 $\pm$ 0.11 a
	L45	0.86 $\pm$ 0.34 a	0.39 $\pm$ 0.49 ab	0.20 $\pm$ 0.39 a	1.57 $\pm$ 1.19 a	0.17 $\pm$ 0.12 a
Year (Y)	2020	0.90 $\pm$ 0.30 a	0.42 $\pm$ 0.50 a	0.27 $\pm$ 0.44 a	1.69 $\pm$ 1.12 a	0.18 $\pm$ 0.11 a
	2021	0.83 $\pm$ 0.37 a	0.35 $\pm$ 0.47 a	0.21 $\pm$ 0.38 a	1.52 $\pm$ 1.14 a	0.17 $\pm$ 0.11 a
Shoot length (L)	< 6 cm	0.70 $\pm$ 0.46 a	0.24 $\pm$ 0.43 a	0.15 $\pm$ 0.35 a	1.12 $\pm$ 1.00 a	0.15 $\pm$ 0.12 a
	8–18 cm	0.96 $\pm$ 0.19 b	0.44 $\pm$ 0.50 b	0.22 $\pm$ 0.41 a	1.72 $\pm$ 1.00 b	0.19 $\pm$ 0.10 b
	>20 cm	0.96 $\pm$ 0.20 b	0.47 $\pm$ 0.50 b	0.34 $\pm$ 0.46 b	1.99 $\pm$ 1.22 b	0.19 $\pm$ 0.11 b
Shoot type (T)	VBS	0.91 $\pm$ 0.28 b	0.07 $\pm$ 0.25 a	0.04 $\pm$ 0.18 a	1.06 $\pm$ 0.55 a	0.13 $\pm$ 0.06 a
	MBS	0.84 $\pm$ 0.37 a	0.70 $\pm$ 0.46 b	0.43 $\pm$ 0.48 b	2.15 $\pm$ 1.29 b	0.23 $\pm$ 0.13 b
ANOVA						
G		ns	*	*	*	*
Y		ns	ns	ns	ns	ns
L		***	***	*	***	**
T		*	***	***	***	***
L $\times$ T		ns	*	ns	*	*

Factor interactions that did not exert a statistically significant effect were omitted from the table.

Number and distribution of catkins on shoots

No shoot with a catkin on the first node was found in any genotype in any year. Shoot length and shoot type had a very highly significant effect on the number of catkins differentiated on the second node; the influence of genotype was significant, while the year did not significantly influence the results (Table 5).

Table 5. Number and distribution of catkins on shoots

Factor	Factor levels	Number of catkins				
		On the 1 st node	On the 2 nd node	On the 3 rd node	Per shoot	Per node
Genotype (G)	L6	0.00 ± 0.00 a	0.22 ± 0.42 a	0.34 ± 0.47 a	3.48 ± 1.59 a	0.40 ± 0.20 a
	L16	0.00 ± 0.00 a	0.43 ± 0.57 b	0.64 ± 0.59 b	4.49 ± 1.99 b	0.56 ± 0.26 b
	L45	0.00 ± 0.00 a	0.28 ± 0.45 a	0.47 ± 0.53 a	3.68 ± 2.24 a	0.44 ± 0.27 a
Year (Y)	2020	0.00 ± 0.00 a	0.33 ± 0.51 a	0.48 ± 0.56 a	3.78 ± 2.01 a	0.46 ± 0.26 a
	2021	0.00 ± 0.00 a	0.32 ± 0.49 a	0.54 ± 0.54 a	4.15 ± 2.12 a	0.50 ± 0.26 a
Shoot length (L)	< 6 cm	0.00 ± 0.00 a	0.50 ± 0.50 b	0.70 ± 0.51 b	3.55 ± 1.27 a	0.50 ± 0.18 ab
	8–18 cm	0.00 ± 0.00 a	0.03 ± 0.48 a	0.55 ± 0.55 b	4.34 ± 1.97 a	0.52 ± 0.32 b
	>20 cm	0.00 ± 0.00 a	0.19 ± 0.48 a	0.28 ± 0.53 a	4.00 ± 2.66 a	0.42 ± 0.30 a
Shoot type (T)	VBS	0.00 ± 0.00 a	0.56 ± 0.56 b	0.72 ± 0.54 b	4.20 ± 2.28 a	0.54 ± 0.29 b
	MBS	0.00 ± 0.00 a	0.10 ± 0.30 a	0.30 ± 0.48 a	3.72 ± 1.80 a	0.42 ± 0.21 a
ANOVA						
G		ns	**	***	**	***
Y		ns	ns	ns	ns	ns
L		ns	***	***	ns	**
T		ns	***	***	ns	**
L × T		ns	***	***	ns	ns

Factor interactions that did not exert a statistically significant effect were omitted from the table.

Shoots of genotype L16 had the highest number of catkins on the second node (0.43), while no significant differences were found for genotypes L6 and L45 (0.22 and 0.28, respectively). Shoots shorter than 6 cm had the highest number of catkins on the second node (0.50), while their number was lowest on shoots 8–18 cm long (0.03). On the second node of VBS, there was an average of 0.56 catkins, while in MBS, the value of this parameter was 0.10. The interaction of shoot length × shoot type had a very highly significant effect on the number of catkins on the second node.

Genotype, shoot length, and shoot type had a very highly significant effect on the number of catkins differentiated on the third node, while the influence of the year was not significant (Table 5). The highest number of catkins on the third node was determined in genotype L16 (0.64), and the lowest in genotype L6 (0.34). The highest number of catkins on the third node was found on shoots shorter than 6 cm (0.70), and the lowest on shoots longer than 20 cm (0.28). In VBS, the number of catkins on the third node was 0.72, while in MBS, it was 0.30. A very highly significant influence on the value of this parameter was exerted by the shoot length × shoot type interaction.

Genotype had a highly significant effect on the number of catkins per shoot, while year, shoot length, and shoot type had no influence (Table 5). Genotype L16 had the highest number of catkins per shoot (4.49), and genotype L6 had the lowest (3.48). On shoots up to 6 cm long, there were highly significantly fewer catkins (3.55) than on shoots 8–18 cm long (4.34) and those longer than 20 cm (4.00). The differences in the number of catkins per shoot between the latter two length categories were not significant. VBS had 4.20 catkins, while MBS had 3.72.

The average number of catkins per node was 0.48. Genotype had a very highly significant effect on this parameter, shoot length and shoot type exerted a highly significant influence, while the year did not significantly influence the results (Table 5). Genotype L16 had the highest number of catkins per node (0.56), and genotype L6 had the lowest (0.40). On shoots up to 6 cm and 8–18 cm long, a higher number of catkins was determined (0.50 and 0.52, respectively) than in the longest shoots (0.42). In VBS, there were 0.54 catkins per node, which was very highly above the value of this parameter in MBS (0.42).

The number of catkins per shoot was in a weak but statistically highly significant negative correlation with shoot thickness ($r = -0.189^{**}$), and in a moderately strong negative correlation with the number of vegetative buds per shoot ($r = -0.641^{**}$) and the number of vegetative buds per node ($r = -0.675^{**}$).

Structure of one-year-old shoots

In the structure of one-year-old shoots, vegetative shoots were the least represented ($1.3 \pm 0.49\%$), with no significant effect of the factors examined (Table 6). The average proportion of staminate shoots was $12.2 \pm 1.41\%$, and their representation decreased significantly with increasing shoot length, from 28.9% in the shortest shoots to 2.8% in shoots longer than 20 cm. The effect of genotype on this trait was also significant. Pistillate shoots accounted for $8.0 \pm 1.17\%$, with a significantly greater representation in genotypes L6 and L45 compared with L16. With increasing shoot length, the proportion of pistillate shoots also increased significantly, which was expressed through a highly significant interaction in VBS longer than 20 cm. Combined shoots were the most represented category ($78.5 \pm 11.77\%$), and their proportion was significantly higher in shoots longer than 8 cm (83.3–85.6%) compared with the category shorter than 6 cm ($66.7 \pm 13.52\%$). For this trait, an interaction was established between shoot length and shoot type, with a significantly higher proportion of combined shoots recorded in MBS longer than 20 cm than in VBS of the same length category. The total proportion of fruiting shoots, including both pistillate and combined shoots, was $86.5 \pm 11.47\%$, with a very significant effect of shoot growth length: shoots longer than 8 cm had a higher proportion of fruiting wood (93.9–96.1%) compared with the shortest category ($69.4 \pm 3.44\%$).

Table 6. Structure of one-year-old shoots

Factor	Factor levels	Vegetative shoots	Staminate shoots	Pistillate shoots	Combined shoots	Fruiting shoots
Genotype (G)	L16	0.0 ± 0.00 a	16.1 ± 3.63 c	4.5 ± 1.97 a	79.4 ± 18.71 a	83.8 ± 7.62 a
	L45	1.92 ± 0.77 a	10.3 ± 3.02 b	9.9 ± 2.05 b	77.9 ± 18.56 a	87.8 ± 13.61 a
	L6	0.0 ± 0.00 a	6.7 ± 2.64 a	7.8 ± 2.84 b	85.5 ± 19.73 a	93.3 ± 2.64 a
Year (Y)	2010	1.7 ± 0.96 a	9.4 ± 2.19 a	8.9 ± 2.13 a	80.0 ± 8.99 a	88.9 ± 12.35 a
	2011	1.7 ± 0.96 a	12.8 ± 2.50 a	7.2 ± 1.93 a	78.3 ± 18.08 a	85.6 ± 12.63 a
Shoot length (L)	< 6 cm	1.7 ± 0.96 a	28.9 ± 4.39 c	2.8 ± 1.23 a	66.7 ± 15.52 a	69.4 ± 13.44 a
	8-18 cm	1.1 ± 0.78 a	5.0 ± 1.63 b	8.3 ± 2.07 b	85.6 ± 17.63 b	93.9 ± 11.79 b
	>20 cm	1.1 ± 0.78 a	2.8 ± 1.23 a	12.8 ± 2.50 c	83.3 ± 17.79 b	96.1 ± 11.45 b
Shoot type (T)	VBS	0.7 ± 0.52 a	7.8 ± 1.63 a	4.4 ± 1.26 a	87.0 ± 18.05 a	91.5 ± 11.70 a
	MBS	1.9 ± 0.82 a	16.7 ± 2.27 b	11.5 ± 1.94 b	70.0 ± 17.79 a	81.4 ± 12.37 a
ANOVA						
G		ns	*	*	ns	ns
Y		ns	ns	ns	ns	ns
L		ns	***	**	*	***
T		ns	ns	**	ns	ns
L × T		ns	ns	***	**	ns

Factor interactions that did not exert a statistically significant effect were omitted from the table.

DISCUSSION

Morphological characteristics of shoots

The average shoot thickness aligns with Solar & Štampar (2003), while slightly higher values reported by Kelc et al. (2010) are likely due to genotypic specificities, tree age, and sampling methods. Interannual variations (greater thickness in 2020) resulted from favourable hydrological conditions during May–July. The significant influence of agro-ecological factors and genotype on secondary thickening is supported by Milošević (1987), Ljubojević et al. (2011), and Milatović et al. (2014), while Kelc et al. (2010) noted that heat and drought reduce walnut shoot thickness. A moderately strong positive correlation obtained in this study confirms the morphological interdependence of thickness and length (Solar et al., 2004).

The average node number correlates with Solar & Štampar (2003) and shows a strong dependence on shoot length and thickness. This is explained by higher photosynthetic activity in shoots with more nodes, ensuring greater assimilate influx for secondary thickening (Kelc et al., 2010). Analysis suggests shoot length depends more on internode elongation than on node number, agreeing with Pallardy (2008) and Bland (1978). Genotype significantly influenced this, with transitional bearing types having more nodes than terminal types (Solar & Štampar, 2003).

The difference in minimum node numbers between VBS and MBS suggests a "critical node number" is required to initiate female inflorescence differentiation (Dennis, 1986; Abbott, 1977). This node count is determined in early summer of the previous year (Janković, 2002). The elongation index showed no significant interannual variations, suggesting a balanced climatic influence on longitudinal and lateral growth. However, a higher index in shoots >20 cm may indicate lower abiotic stress tolerance and weaker assimilate flow (Puntieri et al., 1998). The higher elongation index in VBS compared to MBS ones is due to active apical meristems, whereas MBS terminate growth via apical inflorescences (Bell, 1991; Sabatier & Barthélémy, 2001).

Number and distribution of vegetative buds on shoots

Shoots with a terminal vegetative bud are usually vegetative, as walnut mixed buds are typically located at the apex (Germain et al., 1975; Mičić et al., 1987; Polito, 1998). This study confirmed a low frequency of vegetative buds in the terminal position when mixed buds were present on lower nodes of the fruiting shoot. While genotype did not significantly affect the terminal node, the number of vegetative buds at the subterminal position and per shoot was highest in cultivars with the lowest catkin counts, confirming a moderately strong negative correlation between these two bud types.

Solar & Štampar (2003) highlight the influence of bearing habit, where terminal types possess more vegetative buds compared to lateral genotypes. In our study, genotype and shoot length had a very highly significant effect on this parameter, while year and shoot type had no influence. Kelc et al. (2010) reported that the year and spring temperatures significantly influence this parameter.

The established positive correlation between the number of vegetative buds and both node count and shoot thickness aligns with the findings of Solar et al. (2004). Our results confirm that longer shoots differentiate more vegetative buds per node. A similar phenomenon was observed in apricots (Milatović et al., 2014). Finally, the very strong positive correlation between the number of vegetative buds and female flowers on the first node is directly linked to the predominantly terminal bearing habit of the studied cultivars.

Number and distribution of mixed buds on shoots

The studied genotypes significantly differed in the number of mixed buds per shoot. According to Solar & Štampar (2003), terminal bearing types differentiate one mixed bud, intermediate types 1.1–1.2, and lateral bearing genotypes 1.7. Kelc et al. (2010) reported averages of 1.8 mixed buds for 'Franquette' and 2.0 for 'Fernor'. Although the year of study had no significant effect in our trial, Kelc et al. (2010) highlight the crucial role of environmental factors on walnut fruiting potential.

A higher number of mixed buds was recorded on the first node of VBS compared to MBS, whereas on the second and third nodes, the opposite trend was observed. This suggests that young fruits exert an inhibitory effect on flower induction in the closest buds (first node), while this influence weakens on

lower nodes and in longer shoots with more distant internodes. Such inhibition of differentiation due to the presence and proximity of fruit has been confirmed by numerous authors in apples (Fulford, 1966; Chan & Cain, 1967; Landsberg, 1979; Izadyar, 1997; Neilsen & Dennis, 2000) and pears (Huet, 1973).

All genotypes had a higher total and lateral mixed bud count on MBS than on VBS. In fruiting annual shoots, terminal growth ceases due to the formation of female inflorescences (Mičić, 1992), followed by sympodial growth from lateral vegetative points (Bell, 1991; Sabatier & Barthélémy, 2001). MBS had more nodes and a lower elongation index, because of shorter internodes. A higher node count, shorter internodes, larger diameter, and greater leaf area are factors positively correlated with the number of differentiated mixed buds per shoot, as also indicated by Kelc et al. (2007).

Despite the influence of genotype and shoot type on the top three nodes, the highest number of mixed buds was always located on the terminal node, with a sharp decline towards the shoot base. According to Dzhuvinov & Gandev (2014), genotypes L16 and L45 (with less than 10% lateral buds) belong to the terminal bearing type, while L6 (12–13% lateral buds) is classified as an intermediate bearing type.

Number and distribution of catkins on shoots

Catkin differentiation on the terminal node is rare, as also noted by Mert (2010). The average number of catkins on the second and third node indicates that the majority of catkins differentiate closer to the base of the shoot, which is in accordance with the findings of Germain et al. (1975) and Langrová & Sladký (1971).

A negative correlation was established between catkin differentiation and vegetative activity, which is consistent with the explanation by Ryugo et al. (1985) that reduced vegetative vigour, driven by changes in the carbohydrate-to-nitrogen ratio, promotes the differentiation of a higher number of catkins. Furthermore, the number of catkins is in a moderately strong negative correlation with the number of female flowers on the first node, suggesting that the presence of female flowers inhibits catkin development.

The protandrous genotype (L16) had significantly more catkins than the protogynous ones (L6 and L45). In protandrous genotypes, catkin differentiation in spring is faster and more abundant due to the lack of competition with mixed buds (Ryugo et al., 1985).

Shoots with more nodes and leaves had more female flowers and fewer catkins. In this regard, Huet (1973) emphasises that a sufficient number of leaves is a prerequisite for flower differentiation. The number and distribution of catkins were significantly influenced by the interactions between genotype, year, and shoot type, confirming the complexity of sex differentiation under the influence of internal and external factors (Chailakhyan & Khrianin, 1987).

Structure of one-year-old shoots

The low proportion of vegetative shoots obtained in the present study is consistent with the findings of Apostolova (2008), who reported a similar range. The established negative correlation between the presence of staminate (male) shoots and their vegetative vigour confirms the tendency that increasing shoot length favours the formation of vegetative and mixed buds. The average proportion of combined shoots is also in agreement with the results of Apostolova (2008).

The high proportion of fruiting shoots and the absence of significant annual variations in this study indicate more stable cropping compared to the results of Apostolova (2008), whose data suggest a tendency of the orchard towards biennial bearing. The higher abundance of fruiting shoots in shoots longer than 8 cm, along with a confirmed positive correlation with shoot length, thickness, and node number, is attributed to a better carbohydrate supply due to a larger leaf area, which is crucial for flower initiation (Huet, 1973).

CONCLUSION

In most cases, genotype, shoot length, and shoot type exerted a significant influence on the morphological characteristics of one-year-old shoots, the representation of different bud categories on these shoots, and the representation of various shoot types within the overall structure of one-year-old walnut wood, whereas the effect of the year was not significant.

REFERENCES

- Abbott, D. L. (1977). Fruit bud formation in Cox's Orange Pippin. Long Ashton Research Station, Annual Report for 1976, 167-176.
- Apostolova, M. (2008). Genotypic specificities in the structure of walnut bearing wood in the ecological conditions of Čačak. Proceedings of the National Scientific and Technical Conference with international participation "Ecology and Health", Plovdiv, Bulgaria, 218-224.
- Bell, A. D. (1991). Plant form. An illustrated guide to flowering plant morphology. Oxford University Press, Oxford, UK.
- Bland, J. (1978). A comparison of certain aspects of ontogeny in the long and short shoots of McIntosh apple during one annual growth cycle. PhD Thesis, University Minnesota, St. Paul, USA.
- Chailakhyan, M. K., & Khrianin, V. N. (1987). Sexuality in plants and its hormonal regulation, Springer-Verlag, Berlin, Germany.
- Chan, B. G., & Cain, J. C. (1967). The effect of seed formation on subsequent flowering in apple. *Proceedings of the American Society for Horticultural Science*, 91, 63-68.
- Dennis, F. G. (1986). The physiology of flowering and fruit set in fruit trees. *Proceedings of the Oregon Horticultural Society*, 77, 33-43.
- Dzhuvinov, V., & Gandev, S. (2014). Walnut breeding – modern trends of development. *Journal of Mountain Agriculture on the Balkans*, 17, 176-191.
- Fulford, R. M. (1966). The morphogenesis of apple buds IV. The effect of fruit. *Annals of Botany*, 30(120), 597-606. <http://www.jstor.org/stable/42907924>
- Germain, E., Jalinat, J., & Marchou, M. (1975). Divers aspects de la biologie florale du noyer [Various aspects of the floral biology of the walnut tree]. *Bulletin Technique d'Information*, 282, 1-13. <https://hal.inrae.fr/hal-02858762>
- Huet, J. (1973). Floral initiation in pear trees. *Acta Horticulturae*, 34, 193-198. <https://doi.org/10.17660/ActaHortic.1973.34.25>
- Izadyar, A. B. 1997. Anatomical aspects of alternate bearing in apple trees. *Acta Horticulturae*, 435, 255-260. <https://doi.org/10.17660/ActaHortic.1997.435.25>
- Janković, D. (2002). Genotipske specifičnosti u formiranju ženskog gametofita oraha. [Genotypic specificities in the formation of the female gametophyte in walnut]. Doctoral dissertation. University of Pristina, Faculty of Agriculture, Lešak.
- Kelc, D., Štampar, F., & Solar, A. (2007). Fruiting behaviour of walnut tree influences relationships between morphometric traits of parent wood and nut weight. *Journal of Horticultural Science and Biotechnology*, 82, 439-445. <https://doi.org/10.1080/14620316.2007.11512256>
- Kelc, D., Štampar, F., & Solar, A. (2010). Architectural traits of fruiting shoots in *Juglans regia* (Juglandaceae) related to fruiting habit and environmental conditions. *Australian Journal of Botany*, 58, 141-148. <https://doi.org/10.1071/BT09095>
- Landsberg, J. J. (1979). From bud to bursting blossom: weather and the apple crop. *Weather*, 34, 394-407. <https://doi.org/10.1002/j.1477-8696.1979.tb03382.x>
- Langrová, V., & Sladký, Z. (1971). The role of growth regulators in the differentiation of walnut buds (*Juglans regia* L.). *Biologia Plantarum*, 13, 361-367. <https://doi.org/10.1007/BF02930925>
- Lučić, P., Đurić, G., & Mičić, N. (1996). Voćarstvo I: Biologija voćaka, rasadnička proizvodnja, pomotehnika, podizanje i oržavanje voćnjaka. [Fruit growing I: Biology of fruit trees, nursery production, support techniques, raising and cultivating orchards]. Nolit, Partenon, Institut za istraživanje u poljoprivredi Srbije, Beograd.
- Ljubojević, M., Ognjanov, V., Čukanović, J., & Mladenović, E. (2011). Selekcija slabobujnih podloga za višnju i trešnju. [Selection of low-vigour rootstocks for sour and sweet cherries]. Zbornik radova III savetovanja "Inovacije u voćarstvu", Beograd, pp. 205-214. <https://www.cabidigitallibrary.org/doi/pdf/10.5555/20183065078>

- Mert, C. (2010). Anther and pollen morphology and anatomy in walnut (*Juglans regia* L.). *HortScience*, 45, 757-760. <https://doi.org/10.21273/HORTSCI.45.5.757>
- Mićić, N., Blesić, M., Đurić, G., & Mališević, E. (1987). Anatomsko-morfološke karakteristike zimskih pupoljaka i tipovi rodni grančica oraha (*Juglans regia* L.). [Anatomical and morphological characteristics of winter buds and types of walnut (*Juglans regia* L.)]. fruiting branches. *Poljoprivredni pregled*, 1-3, 19-27.
- Mićić N. 1992. Prilog klasifikaciji generativnih pupoljaka voćaka. [A contribution to the classification of generative buds in fruit trees.] *Jugoslovensko voćarstvo*, 26, 97-98: 3-15.
- Milatović, D., Đurović, D., & Zec, G. (2014). Morfološke osobine rodni grančica sorti kajsije. [Morphological characteristics of fruiting branches in apricot cultivars]. *Journal of Agricultural Sciences*, 59(3), 265-274. <https://doi.org/10.2298/jas1403265m>
- Milatović, D., Nikolić, D., Janković, S., Janković, D., & Stanković, J. (2020). Morphological characteristics of male reproductive organs in some walnut (*Juglans regia* L.) genotypes. *Scientia Horticulturae*, 272, 109-587. <https://doi.org/10.1016/j.scienta.2020.109587>
- Milošević, T. (1987). Uticaj podloga na dinamiku rasta ploda, vegetativnog porasta i procesa diferencijacije generativnih pupoljaka šljive. [The influence of rootstocks on the dynamics of fruit growth, vegetative growth and the process of generative bud differentiation in plum]. Master's thesis. University of Sarajevo, Faculty of Agriculture and Food Sciences.
- Milošević, T. (1993). Uticaj podloge na vegetativni razvoj sorti šljive. [The influence of rootstock on the vegetative development of plum cultivars]. *Poljoprivreda i šumarstvo*, 39(3-4), 33-41.
- Neilsen, J. C., & Dennis, F. G. (2000). Effects of seed number, fruit removal, bourse shoot length and crop density on flowering in 'Spencer Seedless' apple. *Acta Horticulturae*, 527, 137-146. <https://doi.org/10.17660/ActaHortic.2000.527.15>
- Pallardy, S. G. (2008). Physiology of woody plants, third edition. Elsevier, London, UK.
- Polito, V. S. (1998). Floral biology: flower structure, development, and pollination. In: Ramos, D.E. (ed.), Walnut production manual. University of California, Oakland, USA, pp. 127-132.
- Puntieri, J., Barthélémy, D., Martinez, P., Raffaele, E., & Brion, C. (1998). Annual-shoot growth and branching patterns in *Nothofagus dombeyi* (Fagaceae). *Canadian Journal of Botany*, 76, 673-685. <https://doi.org/10.1139/b98-041>
- Republic Hydrometeorological Service of Serbia, (2021). Available online: <http://www.hidmet.gov.rs/> (accessed on 15 January 2026).
- Ryugo, K., Bartolini G., Carlson, R. M., & Ramos, D. E. (1985). Relationship between catkin development and cropping in the Persian walnut 'Serr'. *HortScience*, 20, 1094-1096. <https://doi.org/10.21273/HORTSCI.20.6.1094>
- Sabatier, S., & Barthélémy, D. (2001). Bud structure in relation to shoot morphology and position on the vegetative annual shoots of *Juglans regia* L. (Juglandaceae). *Annals of Botany*, 73, 13-22. <https://doi.org/10.1006/anbo.2000.1312>
- Solar, A., & Štampar, F. (2003). Genotypic differences in branching pattern and fruiting habit in common walnut (*Juglans regia* L.). *Annals of Botany*, 92, 317-325. <https://doi.org/10.1093/aob/mcg137>
- Solar, A., Hudina, M., & Štampar, F. (2004). Fruiting habit and branching pattern affect vegetative growth and reproductive ability in walnut (*Juglans regia* L.). *Acta Horticulturae*, 663, 387-392. <https://doi.org/10.17660/ActaHortic.2004.663.66>
- Stanković, J., Ralević, R. N., & Ljubanović-Ralević, I. (2002). Statistika sa primenom u poljoprivredi. Poljoprivredni fakultet, Beograd-Zemun.

Agrisolar - Application of photovoltaic technologies in agriculture

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Abstract

Agrivoltaic, or agrisolar, systems represent an integrated approach to the application of photovoltaic (PV) technologies in agriculture, enabling simultaneous electricity generation and agricultural production on the same land area. This paper reviews the main agrisolar technologies and design options, including elevated and interspace PV concepts, systems for permanent crops and pastures, greenhouse-integrated PV and rooftop PV on agricultural buildings. Agrisolar systems are highlighted for their benefits in supporting irrigation, modifying microclimates, reducing crop heat stress, and supplying energy for frost protection. The paper also discusses the main engineering trade-offs related to land use, structural design and maintenance on one hand as well as shading and supply reliability on the other. In addition to the general review, an illustrative sizing example is presented for a 20 ha apple orchard with annual electricity demand of about 120–128 MWh. Three supply concepts are compared: a 110 kWp on-grid PV system, a fully autonomous off-grid PV-battery system and a hybrid off-grid system combining PV, limited battery storage and diesel-generator support. For the analysed case, the 110 kWp on-grid system yields about 129.58 MWh/year of PV electricity with a simple payback of about 8.1 years, whereas the hybrid concept reduces the payback to about 5 years under similar agricultural assumptions. By contrast, the fully autonomous battery-based off-grid solution becomes economically unattractive, with an estimated simple payback of about 50 years. The results indicate that the on-grid concept is well suited for reducing electricity costs and maximizing daytime self-consumption, while the fully autonomous off-grid solution becomes economically unfavorable when rare but remarkably high frost-protection loads must be covered. Under such conditions, the hybrid concept provides the most balanced technical and economic solution, because it combines renewable energy use during normal operation with reliable backup for critical short-term peak loads. Agrisolar systems can therefore be regarded as a flexible and multifunctional framework linking energy efficiency, agricultural resilience and climate-adaptive farm infrastructure.

Keywords: agrivoltaics, photovoltaics, agriculture, irrigation, frost protection

INTRODUCTION

Agrisolar, or agrivoltaic, systems combine PV power generation and agricultural production on the same land area. The concept emerged as a response to land-use competition between energy infrastructure and food production and has since evolved into a broader framework for climate-resilient and resource-efficient farming (Dupraz et al., 2011; Weselek et al., 2019; Toledo & Scognamiglio, 2021). Beyond electricity generation, agrisolar systems are increasingly recognized as multifunctional farm infrastructure capable of supporting both energy and agricultural objectives within the same production space.

In practical terms, the importance of agrisolar systems extends beyond renewable power supply. On the energy side, they can reduce electricity costs through on-site self-consumption, contribute to a more stable long-term energy cost structure and reduce dependence on grid electricity. In hybrid configurations, they may also help reduce peak loads and improve the reliability of electricity supply for agricultural consumers such as irrigation pumps, ventilation systems, sensors and automated control units.

At the same time, agrisolar systems may provide important agronomic and environmental benefits. Controlled shading can reduce excessive solar exposure, lower evapotranspiration and mitigate crop heat stress, while the structural integration of PV modules may offer partial protection against hail and heavy rainfall. Such effects can create a more favourable microclimate for selected crops and can be especially valuable when combined with smart irrigation strategies and precision agriculture tools. Previous studies have shown that the agronomic response to agrivoltaic operation strongly depends on local climate, crop sensitivity to shading, system geometry and operational strategy, which means that the benefits of agrisolar systems must be evaluated through an integrated technical and agronomic perspective (Barron-Gafford et al., 2019; Weselek et al., 2021).

From a farm-management perspective, agrisolar systems can also improve the economic and strategic resilience of agricultural enterprises. In addition to direct savings achieved through self-consumption, they may provide additional income through the export of surplus electricity where regulatory and market conditions permit. They can also increase resilience to grid interruptions by supporting backup supply for critical loads, reduce the carbon footprint of production and improve overall land-use efficiency through dual-use operation. In general, the economic performance of such systems depends strongly on the share of PV electricity consumed directly on-site, the value of exported electricity, as well as the associated operation and maintenance costs. Therefore, the key engineering question is not whether agrisolar systems are universally beneficial, but under which technical conditions they can improve the joint performance of energy and agricultural subsystems.

Despite their multiple technical and agronomic advantages, agrisolar systems also involve important trade-offs that must be carefully considered before investment. Compared with conventional ground-mounted PV installations, such systems typically require higher initial capital costs and more complex design processes, since agronomic requirements, structural stability, electrical engineering and agricultural mechanization must be addressed simultaneously. Additional challenges include the risk of inappropriate shading, which may reduce crop yield or product quality, limited access for cleaning and maintenance during the growing season, and regulatory constraints related to grid connection, land use and operational safety. For this reason, successful implementation usually depends on preliminary shading analysis, phased deployment through pilot plots, identification of critical electrical loads and detailed assessment of structural loads, corrosion protection, and long-term operation and maintenance arrangements.

The paper is organized as follows. After the Introduction, the second section reviews the main agrisolar technologies and design options, with emphasis on representative structural typologies and PV system concepts for agricultural applications. The following section presents typical agrisolar applications, including irrigation support, heat-stress mitigation, frost-response support, and rooftop PV use in agricultural storage and drying facilities. The paper then discusses practical implementation considerations through an illustrative sizing example for a 20 ha apple orchard, comparing on-grid, fully autonomous off-grid, and hybrid PV supply concepts from both the technical and economic perspectives. Finally, the main findings are summarized in the Conclusion.

AGRISOLAR TECHNOLOGIES AND DESIGN OPTIONS

Agrisolar systems can be implemented through a broad range of structural and functional concepts, depending on the category of agricultural land, the production system and the required level of integration between farming activities and PV electricity generation, as illustrated in Figure 1. In arable farming, one common approach involves elevated PV structures that provide sufficient clearance for cultivation, machinery passage and routine field operations below the modules. Another concept is based on interspace installation, where PV elements are positioned between productive zones to reduce interference with crop management while still enabling on-site electricity production. These solutions

are particularly relevant where land-use efficiency is a major design objective and where the balance between shading and agricultural productivity must be carefully controlled.

In permanent crop systems, including orchards, vineyards, and other perennial plantations, agrisolar integration requires additional adaptation to row spacing, canopy geometry, crop sensitivity to shading, and access for pruning, spraying and harvesting operations. In such applications, PV structures may serve not only as an energy source, but also as an element that influences the crop microclimate. As indicated in Figure 1, similar integration principles can also be applied to permanent meadows and pastures, where PV systems may coexist with livestock grazing or forage production. In these cases, the land remains agriculturally active while simultaneously contributing to renewable electricity generation, thus improving the overall utilization of available space.

The agrisolar concept extends beyond open-field production. PV modules can also be integrated with elevated greenhouse structures or installed on farm buildings, including storage facilities, barns, and other agricultural infrastructure. Although these solutions differ from field-based dual-use systems, they are an important part of agrisolar development because they strengthen the connection between agricultural production and local renewable energy supply. Overall, the typological diversity presented in Figure 1 confirms that agrisolar should be understood as a flexible and site-specific framework rather than as a single standardized technical solution.

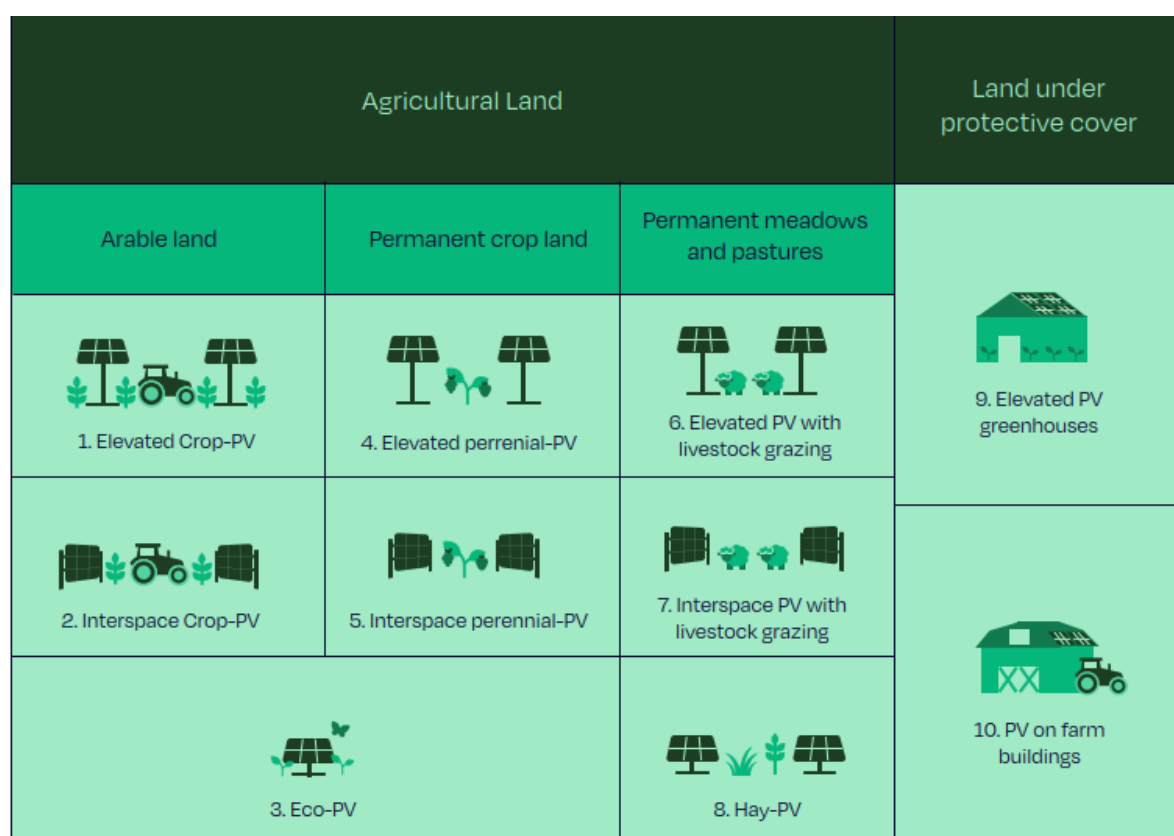


Figure 1. Overview of representative agrisolar typologies for different categories of agricultural land and protected agricultural structures

Table 1 summarizes the preliminary suitability of selected fruit crops for the main agrisolar typologies presented in Figure 1. The classification is based on currently available literature on agrivoltaic performance in fruit crops, berry-crop shade response, vineyard agrivoltaics, and the established use of protective net structures in orchards and berry production. It should be understood as a design-oriented typological guide rather than as a universal agronomic ranking, because the final suitability depends on local climate, cultivar, canopy architecture, row spacing, mechanization requirements, and the acceptable level of shading.

As shown, the most directly compatible typology for tree-fruit crops is the elevated perennial-PV/orchard concept, especially when the PV structure can be integrated with anti-hail, anti-rain, or photoselective protection systems. By contrast, raspberries and strawberries are particularly attractive for protected-cropping agrisolar solutions, because tunnel or greenhouse-type systems can simultaneously provide crop shelter and renewable electricity generation. Vineyard systems occupy an intermediate but highly promising position, since row-based canopy geometry allows technically elegant agrivoltaic layouts with relatively low interference with cultivation practices.

Table 1. Preliminary matching of selected fruit crops with agrisolar typologies

Crop	Main suitable agrisolar typology	Suggested suitability	Short technical note
Apple	Elevated perennial-PV / orchard systems	High	Particularly suitable where the AV structure can also fulfil anti-hail / photoselective protection functions while preserving pruning, spraying and harvest access.
Vineyard	Elevated perennial-PV / vineyard systems; row-integrated or inter-row PV layouts	High	Row geometry and canopy training make vineyard-compatible AV layouts especially attractive, including low-shading row-based concepts.
Raspberry	Elevated over-row AV; PV tunnel / protected berry systems	High	A strong candidate where AV can replace plastic tunnel covers and simultaneously provide weather protection and electricity generation.
Strawberry	PV tunnel / greenhouse-integrated systems; semi-transparent elevated AV	Medium to high	More suitable in protected cultivation than in heavily shaded open-field systems; light distribution and module transparency are critical.
Sweet cherry	Elevated perennial-PV / orchard systems	Medium to high	Particularly relevant when the AV structure is combined with anti-rain / anti-hail protection because of fruit cracking and weather sensitivity.
Sour cherry	Elevated perennial-PV / orchard systems	Medium	Orchard-type AV is feasible by analogy with sweet cherry and other stone-fruit systems, but direct AV evidence is still limited.
Apricot	Elevated or dynamic perennial-PV / orchard systems	Medium to high	Promising especially where late frost, excessive irradiation, drought and heat stress are important design drivers.

Figure 2 illustrates four representative agrisolar system configurations: top left – Elevated Crop-PV/Interspace Crop-PV; top right – PV with livestock grazing; bottom left – elevated perennial-PV/orchard systems; bottom right – PV greenhouses. As shown in Figure 2, these configurations illustrate the broad applicability of agrisolar systems across different agricultural environments and production practices. Their structural diversity reflects the need to adapt PV integration to crop type, land use, mechanization requirements, and the desired level of interaction between energy generation and agricultural production.



Figure 2. Representative agrisolar system configurations: arable land, permanent crops, pastures, and protected agricultural structures: top left Elevated Crop-PV / Interspace Crop-PV, top right PV with livestock grazing, bottom left elevated perennial-PV/orchard systems, bottom right PV greenhouses

The design of PV systems in agrisolar applications cannot be reduced to a single standard configuration, because agricultural consumers differ substantially in terms of load profile, seasonal operation, supply reliability requirements and accessibility of the public electricity network. In contrast to conventional commercial or residential PV projects, agrisolar installations often serve technically diverse loads, including irrigation pumps, ventilation units, sensors, control devices, refrigerated storage auxiliaries, anti-frost equipment and mobile or distributed consumers in orchards and fields. For that reason, the selection of the system concept must be based not only on annual energy balance, but also on the temporal coincidence between solar generation and electrical demand, the acceptable level of supply interruption and the economic consequences of undersized or oversized storage and backup capacity. In the literature on agrivoltaics and agricultural PV integration, system design is increasingly treated as a multidisciplinary problem involving land use, crop response, structural layout, and electrical architecture rather than as a simple question of PV module installation (Toledo & Scognamiglio, 2021; Al Mamun et al., 2022).

The most straightforward concept is the conventional on-grid PV system, which is appropriate when a reliable utility connection already exists and when the farm has daytime electricity demand that can absorb a meaningful share of PV production. In this configuration, the PV generator supplies local agricultural loads first, while the surplus energy is exported to the grid through a grid-tied inverter. This concept is particularly suitable for irrigation during sunny periods, ventilation in livestock or storage facilities, auxiliary consumption in cold rooms and dryers, and digital farm infrastructure such as monitoring and automation systems. Its principal advantages are relatively low capital cost, simple architecture, high conversion efficiency and the absence of large battery storage, which remains one of the most expensive and degradation-sensitive components in autonomous systems. In engineering terms, the attractiveness of the on-grid concept lies in the fact that the grid behaves as a virtual balancing resource: when PV generation exceeds demand excess power is exported, and when local consumption exceeds generation the deficit is imported. However, this configuration also has an important limitation in agricultural practice, namely that standard grid-connected inverters disconnect during grid outages for anti-islanding protection,

which means that a typical on-grid agrisolar system does not ensure continuity of operation for critical farm loads during disturbances unless additional backup architecture is provided.

A second concept is the off-grid PV system with battery storage, which is used when grid access is unavailable, too distant, or economically unjustified. In this case, the PV array, charge controller, inverter and battery bank must be dimensioned so that the system can maintain acceptable supply reliability under variable solar conditions and fluctuating agricultural demand. Such systems are technically suitable for isolated irrigation points, telemetry stations, electric fences, remote valves, small pumps, water treatment units and other distributed agricultural consumers with relatively predictable load levels. Their main benefit is electrical autonomy, which can be highly valuable in remote production areas. Nevertheless, off-grid design becomes significantly more demanding when the agricultural load is both seasonal and dynamic, because the storage system must absorb short-term PV fluctuations while also maintaining sufficient reserve during low-irradiance periods. Reviews of off-grid PV–diesel–battery systems show that multiple configurations are possible, including AC-coupled, DC-coupled and hybrid DC–AC architectures, while the optimal solution depends strongly on the AC load profile and the control strategy of the system components (Salas et al., 2015). In practical agricultural applications, a fully battery-based off-grid concept may therefore become expensive and suboptimal when the design must accommodate rare but very high-power peaks. Large battery banks raise investment cost, replacement cost and thermal-management requirements, while much of the installed storage may remain underutilized for most of the year.

For that reason, a particularly relevant architecture in agrisolar engineering is the hybrid PV system intended to operate primarily off grid, but with minimal battery capacity and auxiliary diesel electric generation (DEG) for exceptional high-power events. This concept is especially rational when the farm has a combination of modest regular loads and rare critical loads with very high instantaneous demand. In such a system, the PV field covers the base daytime demand and contributes to the annual energy balance; a relatively small battery bank is used for control power, short-duration stabilization, black-start capability and supply of smaller loads during short irradiance deficits; while the DEG is reserved for those operating states in which reliability is more important than maximizing renewable fraction. From the standpoint of hybrid-system literature, such architectures are often preferable to diesel-only supply because hybridization improves sustainability and can reduce fuel use, while still maintaining the dispatchability and robustness that purely renewable autonomous systems often lack (Olabode et al., 2021). In addition, experimental studies of PV/diesel systems without battery storage have shown that PV contribution can improve system operation under appropriate load conditions and that reliable design requires the DEG to be sized with respect to peak load rather than average annual consumption (Yamegueu et al., 2011). These findings are directly relevant to agriculture, where many critical loads are not energy-intensive on an annual basis, but are power-intensive during short operational windows.

A highly illustrative example is frost-protection operation with wind machines in orchards. Wind machines are used during radiation frost events to mix warmer air aloft with colder air near the crop surface, and their effectiveness depends on inversion conditions; they must be started before the crop temperature drops below the critical threshold (Snyder, 2000). In practice, these machines are not operated continuously throughout the year, but only during limited frost-risk periods, often on a small number of nights. Even so, they impose substantial short-term electrical or engine-driven power demand. This operating pattern creates a classic mismatch for a purely battery-based off-grid PV concept: the annual energy consumption of the anti-frost system may be relatively modest, yet the instantaneous power requirement during a frost event can be very high. Designing a stand-alone PV-battery system to supply such a load without auxiliary generation would require disproportionate oversizing of both the PV array and the storage bank, leading to low annual utilization of the installed capacity and poor economic efficiency. In contrast, a hybrid architecture with PV, limited batteries and a DEG allows the renewable subsystem to serve regular farm loads during most of the year, while the generator is activated only when the anti-frost equipment must operate or when prolonged low-solar conditions threaten reliability. From a techno-economic perspective, this reduces the need for excessive battery capacity while preserving operational security for critical seasonal events.

Therefore, in agrisolar applications the choice between on-grid, fully off-grid, and hybrid PV concepts should be made according to the structure of agricultural demand rather than according to PV generation

alone. Where a stable grid is present and compensation conditions are acceptable, on-grid systems usually provide the simplest and most economical solution. Where the site is remote and the loads are moderate and predictable, off-grid PV with batteries can offer full autonomy. However, where the installation must support rare but strategically important peak loads—such as frost-protection wind machines, high-power pumps, or emergency ventilation—a hybrid concept based on PV generation, limited battery storage, and auxiliary diesel support is often the most technically justified option. Such a system preserves the decarbonization benefits of solar energy for everyday operation, avoids overinvestment in batteries, and ensures that critical agricultural processes remain protected during short-duration but high-consequence events. In that sense, hybrid agrisolar power architecture should be viewed not as a compromise, but as a targeted engineering response to the distinctive intermittency and risk structure of agricultural electricity demand.

TYPICAL AGRISOLAR APPLICATIONS

One of the most consistently reported benefits of agrisolar systems is their influence on microclimate and water balance. Measurements in dryland and pasture conditions showed that PV shading can preserve higher soil moisture and improve water-use efficiency, while research on irrigated lettuce demonstrated lower crop water consumption under agrivoltaic operation with implications for irrigation scheduling (Hassanpour Adeg et al., 2018; Elamri et al., 2018).

In temperate organic crop rotations, agrivoltaic operation was associated with reduced photosynthetically active radiation, lower summertime soil temperature and crop-specific yield responses, confirming that the effect of shading is neither universally positive nor universally negative, but strongly crop and season dependent (Weselek et al., 2021). From an engineering standpoint, this means that agrisolar is best suited to crops and management schemes that can exploit moderated heat load, reduced evapotranspiration, or improved soil water retention.

The energy side of the concept is equally important. PV-powered irrigation can supply pumps, valves, sensors, telemetry units and automated controllers directly in the field, reducing dependence on long feeders or diesel-only operation. Reviews of solar irrigation systems emphasize that the reliability of agricultural water pumping improves when PV generation is combined with either battery storage, hydraulic storage, or intelligent scheduling of pumping periods according to water demand and solar availability (Wazed et al., 2018; Calero-Lara et al., 2021).

Table 2 summarizes representative agrisolar applications in irrigation support, heat-stress mitigation and frost-response operation, together with their PV-supported functions, operational benefits, and key engineering requirements.

Table 2 Representative agrisolar applications in irrigation support, heat-stress mitigation, and frost-response operation

Application	PV-supported function	Operational benefit	Main engineering requirement
Irrigation pumping	Power supply for pumps, valves, controllers, and telemetry	Reduced diesel dependence and decentralized water delivery	Matching PV generation with hydraulic demand and storage
Heat-stress reduction	Controlled shading of crop canopy	Lower evaporative demand and moderated soil temperature	Crop selection and layout tuned to radiation tolerance
Frost response	Energy support for fans, sprinklers, heaters, and sensors	Faster deployment of active protection measures	Backup supply for night and pre-dawn operation

The contribution of agrisolar systems to frost protection should be interpreted carefully. PV modules alone cannot guarantee prevention of radiation frost or advective freeze events, because frost damage depends on local inversion, wind, humidity, topography and crop phenological stage. Accordingly, agrisolar should not be presented as a standalone anti-frost technology.

Its role is primarily enabling and system-level. The structures may slightly influence night-time radiative exchange locally, but their more important value is that they can provide locally generated electrical power for active frost-protection equipment. Recent reviews classify the main active techniques as air disturbance, sprinkler irrigation and heating, all of which require reliable energy or water supply and careful operational control (Liu et al., 2025).

In orchards, vineyards, or high-value horticultural systems, agrisolar can therefore be integrated with frost-warning sensors, control units, pumps, or electric or hybrid heating devices. This is especially relevant where frost risk is episodic, grid quality is weak, or irrigation and frost protection must be combined within the same farm infrastructure.

Although these systems do not belong to the dominant field-based agrivoltaic concept, an important and highly practical segment of agrisolar application in agriculture is related to use of PV-generated electric energy for suppling cold storages, refrigerated chambers, and crop dryers electric loads. In such cases, PV panels are most often installed on the roofs of existing agricultural buildings and operate as grid-connected rooftop systems that support energy-intensive post-harvest processes. This approach is particularly relevant because cold storage facilities and drying systems represent major electricity consumers in modern agricultural value chains. Refrigeration chambers require continuous or cyclic operation of compressors, evaporator fans, pumps, control equipment, and auxiliary ventilation, while dryers demand substantial electrical input for air handling, circulation, automation, and, in some configurations, electrically assisted heating. Since a significant share of these loads occur during daytime hours, rooftop PV generation can be effectively matched with on-site consumption, increasing self-consumption rates and reducing operating costs. Integration is especially beneficial in facilities with seasonal but intensive use, such as fruit and vegetable cold stores or grain and seed drying plants, where energy demand strongly affects product competitiveness and storage economics. From a technical standpoint, these systems are simpler to implement than elevated agrivoltaic structures because they use existing roofs, standard mounting equipment, and conventional grid-connected inverter configurations. At the same time, they preserve valuable agricultural land and can improve the overall energy performance of the facility without interfering with primary production activities. In broader agrisolar planning, rooftop PV on cold storages and dryers should therefore be considered as an important complementary category, particularly for farms and agri-food enterprises seeking immediate, technically mature, and economically justified decarbonization measures. Although such solutions are structurally different from dual use agrivoltaic systems above crops, they contribute to the same strategic objective: stronger integration of renewable energy into agricultural production, processing, and storage infrastructure.

PRACTICAL IMPLEMENTATION CONSIDERATIONS

On-grid, fully autonomous off-grid and hybrid concepts

To illustrate the practical dimensioning procedure and the justification of the proposed agrisolar concept, an example for a 20 ha apple orchard system was analysed. It should be emphasized that this example is illustrative only, while a real project requires a site-specific agrometeorological assessment and a detailed hydraulic design of the irrigation and frost-protection subsystems. The purpose of the example is to show how the load structure determines the selection of the PV supply concept and why the same annual energy demand can lead to very different technical solutions depending on the required operating mode.

The starting point of the analysis is the identification of the main electrical consumers. The installed loads considered in the example are as follows: automation and control equipment of approximately 2–4 kW, irrigation and fertigation equipment of about 120 kW, anti-frost sprinkling pumps of about 140 kW, and electric wind machines for frost protection with an installed power in the range of 300–375 kW. However, installed power alone is not sufficient for proper dimensioning. For system sizing, the

more relevant quantities are the simultaneous power demand and the annual operating time of each group of consumers. In the analysed case, the effective operation was assumed to be approximately 0.3 kW for automation for 8760 h/year, 120 kW for irrigation and fertigation for 900 h/year, 140 kW for anti-frost sprinkling for 40 h/year, and 300 kW for wind machines for 40 h/year. Under these assumptions, the annual energy demand is about 120 MWh, while the assumed specific PV yield is 1178 kWh/kWp per year (average for Novi Sad region).

Table 3. Input data used for the 20 ha apple orchard system sizing example

Parameter	Value
Automation installed power	2–4 kW
Automation effective demand	0.3 kW
Automation annual operating time	8760 h/year
Irrigation and fertigation power	120 kW
Irrigation and fertigation annual operating time	900 h/year
Anti-frost sprinkling pump power	140 kW
Anti-frost sprinkling annual operating time	40 h/year
Wind-machine power	300–375 kW
Wind-machine effective demand in analysis	300 kW
Wind-machine annual operating time	40 h/year
Estimated annual electricity demand	about 128 MWh/year
Assumed specific PV yield	1178 kWh/kWp/year

The load structure is highly non-uniform over the year. Most of the annual energy consumption is associated with irrigation and fertigation, which occur mainly during the vegetation period and therefore have a relatively favourable temporal overlap with solar production. Using the assumed operating times, irrigation and fertigation account for about 108 MWh/year, automation for about 2.628 MWh/year, anti-frost sprinkling for about 5.6 MWh/year, and wind machines for about 12 MWh/year, which in sum gives about 120 MWh/year. This result is important because it shows that the system is not dominated by one large continuous consumer, but rather by a combination of seasonal daytime loads and rare high-power frost-protection loads. That distinction is crucial when comparing on-grid, fully autonomous off-grid, and hybrid concepts.

On-grid PV system

The first concept considered is the on-grid PV system, which is the simplest solution when a reliable utility connection is available. Based on the assumed specific production of 1178 kWh/kWp/year, the data indicate that a PV plant of about 110 kWp (or approximately 100 kW on the inverter side) is sufficient to cover the considered annual demand. The expected annual PV generation is 129.58 MWh, of which 96.69 MWh/year is self-consumed on site, while 32.89 MWh/year is exported to the grid. The corresponding investment cost is estimated at 113850€, annual operation and maintenance costs at 1708€/year, and the resulting net annual benefit at 14131€/year, giving a simple payback of about 8.1 years.

The monthly production-versus-consumption graph (Figure 3) for the on-grid case clearly supports this result. It shows that electricity demand is concentrated in the period from spring to early autumn, especially during irrigation months, while PV production also reaches its maximum during the same part of the year. This seasonal coincidence improves self-consumption and makes the on-grid option technically attractive and economically efficient for covering the dominant annual energy demand. At

the same time, the graph also reveals the main limitation of the on-grid concept: although annual energy coverage is satisfactory, this solution does not inherently guarantee autonomous supply during grid outages or during short but critical nighttime frost events. Therefore, on-grid PV is highly suitable for reducing annual electricity costs, but not by itself for fully securing frost-protection operation.

Fully autonomous off-grid system

The second concept is a fully autonomous off-grid system without a DEG. In this case, the critical design condition is not the annual energy consumption, but the requirement to cover a nighttime frost-protection scenario of around 450 kW for 4 h. To satisfy that operating mode, a PV plant of about 2 MWp would be required, together with a battery system of at least 2 MWh. In the economic estimate presented in the slides, the capital expenditure is based on a 2 MWp PV system, PV support structure costs of around 900€/kWp, BOS costs of 15%, a 3 MWh BESS at roughly 220€/kWh, and additional costs for PCS, switchgear, EMS, and reserve. As a result, the total investment reaches approximately 2,8–3 million €. Under the assumptions of apple yield, selling price and avoided frost-related loss adopted, the simple payback is about 50 years, indicating that a fully battery-supported off-grid solution is technically possible but economically unfavourable for this application.

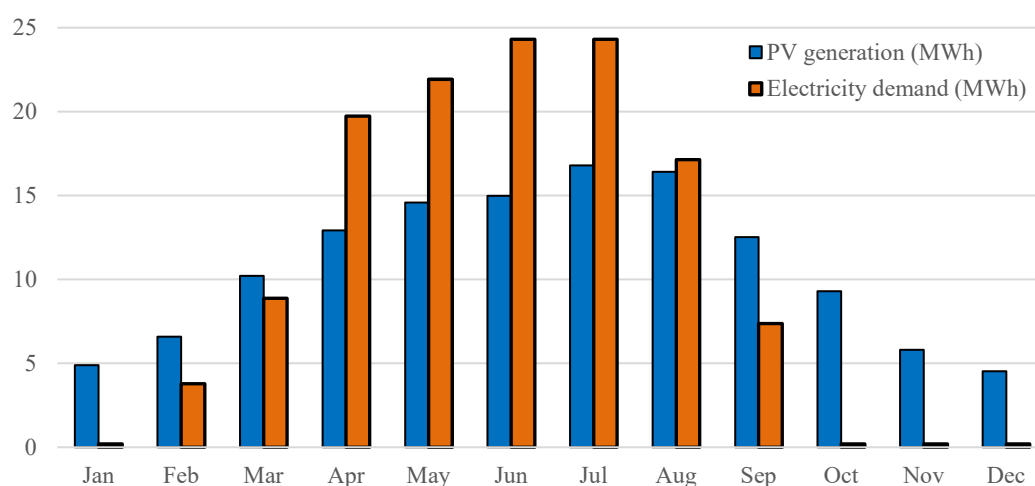


Figure 3. Monthly PV generation vs. electricity demand of 20 ha apple orchard system and 110kWp Agrivoltaic systems

Hybrid off-grid system

The third concept is the hybrid off-grid system with diesel-generator support, which is the most rational solution for the considered orchard case. In this configuration, the PV system is not designed to carry the full frost-protection peak demand autonomously. Instead, a 600 kW diesel generator is proposed, with a relatively small battery of about 30 kWh and a 110 kWp PV system whose role is to reduce diesel runtime and cover the regular daytime energy demand wherever possible. The total investment in this case is estimated at approximately 0.3 million € and the simple payback is about 5 years under the same agricultural and economic assumptions used for the other scenarios. The engineering logic behind this result is straightforward: using PV and a small battery to support regular operation is much more cost-effective than oversizing both the PV field and the storage system for several rare frost nights per season.

Analysis results

Overall, the analysed example shows that the most appropriate agrisolar supply concept depends not only on the annual energy balance, but primarily on the power profile, seasonality and reliability requirement of the agricultural loads. For reducing electricity bills and maximizing daytime self-consumption, the 110 kWp on-grid PV option is a well-matched solution. For complete nighttime autonomy during rare but very high frost-protection loads, a pure off-grid PV-battery system becomes

disproportionately expensive. For orchards with critical anti-frost equipment such as wind machines, the most balanced solution is therefore the hybrid architecture, in which PV covers the base and seasonal daytime demand, the battery provides only limited short-term support and the DEG generator supplies rare high-power emergency or frost-protection events. This configuration offers the best compromise between investment cost, operational reliability and practical agricultural functionality.

Table 4. outlines the comparison of the analysed supply concepts: on-grid, off-grid without diesel support and hybrid off-grid with diesel support.

Table 4. Comparison of the analysed agrisolar supply concepts

Parameter	On-grid PV system	Off-grid PV, battery without DEG	Off-grid PV Hybrid PV, battery plus DEG
PV size	110 kWp	2.0 MWp	110 kWp
Inverter / auxiliary source	100 kW inverter	autonomous operation	600 kW DEG
Battery capacity	–	2–3 MWh	30 kWh
Annual (useful) PV generation	129.58 MWh/year	128 MWh/year	111 MWh/year
Self-consumption	96.69 MWh/year	96.69 MWh/year	PV used to reduce DEG runtime
Export to grid	32.89 MWh/year	no	no
CAPEX	~113850 €	~2.8–3.0 million €	~ 300000 €
O&M	1708€/year	40000€/year	6500€/year
Simple payback	8.1 years	about 50 years	about 5 years

The difference in the estimated payback periods between the on-grid and the hybrid PV–DEG system results from the fact that the two concepts generate economic benefits through different mechanisms. In the on-grid case, the payback of 8.1 years is based on the annual net financial effect obtained from electricity savings through self-consumption, revenue from surplus electricity exported to the grid, and annual operation and maintenance costs. According to the analysed case, the 110 kWp on-grid system requires an investment of 113850 €, while the net annual benefit is 14131 €/year, which directly gives a simple payback of about 8.1 years. In contrast, the hybrid PV–DEG concept is not justified primarily by electricity savings, but by the avoidance of frost-related apple losses. For that system, the total investment is about 300000 €, while the annual economic effect was estimated at approximately 65000 €/year, based on the assumed orchard yield, apple market price and avoided frost damage. This results in a simple payback of approximately 5 years. Therefore, although the hybrid system is more expensive, its economic justification is stronger because it protects agricultural revenue rather than only reducing energy costs.

Sensitivity analysis

A simplified sensitivity analysis was performed to examine the influence of electricity and diesel-fuel prices on the economic results of the analysed supply on-grid and hybrid concepts.

For the on-grid PV case, the base-case electricity import and export price is assumed to be equal, $p_{el}=0.122$ €/kWh. Under this assumption, the annual benefit can be written in the simplified form

$$B_{on} = (E_{sc} + E_{exp}) \cdot p_{el} - C_{O\&M,pv} = E_{PV} \cdot p_{el} - C_{O\&M,pv}$$

where $E_{PV}=129.58$ MWh/year is the total annual PV generation (E_{sc} self-consumption, E_{exp} system exported energy) and $C_{O\&M,pv}=1708$ €/year. As already presented, this gives a base-case annual net benefit of approximately 14.1 k€/year and a simple payback of about 8.1 years. To assess price sensitivity, the electricity price was varied by $\pm 20\%$ around the base value. The results show that the simple payback

changes from about 10.36 years at 0.098 €/kWh to about 6.61 years at 0.146 €/kWh, which confirms that the economic performance of the on-grid concept is strongly dependent on the electricity price.

Figure 4 shows the sensitivity of the on-grid PV system payback to electricity price variation.

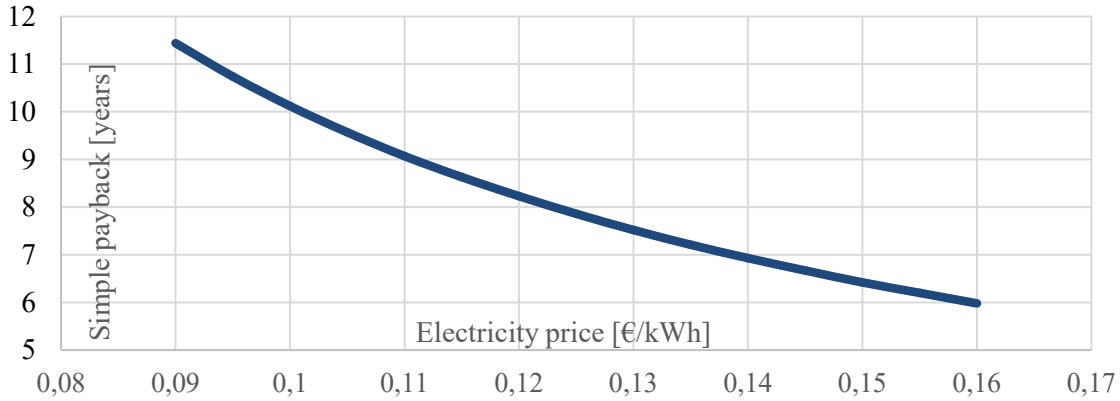


Figure 4. Sensitivity of the on-grid PV system simple payback to electricity price variation

For the hybrid PV–DEG concept, a simplified parametric model was used in which the annual economic effect depends on the value of PV electricity and the cost of diesel consumption:

$$B_{hy} = B_{hy,0} + E_{PV,hy}(p_{el} - p_{el,0}) - F_{ann}(p_d - p_{d,0}) - C_{O\&M,hy}$$

where $B_{hy,0} = 65$ k€/year is the base-case annual economic effect, $E_{PV,hy} = 111$ MWh/year is the annual PV generation of the hybrid system, $p_{el,0} = 0.122$ €/kWh is the base electricity price, $p_{d,0} = 1.30$ €/L is the assumed base diesel price, and $F_{ann} = 4752$ L/year is the assumed annual diesel consumption during limited frost-protection and emergency operation and $C_{O\&M,hy} = 6500$ €/year. Under these assumptions, the hybrid-system simple payback is about 5.13 years in the base case. When annual diesel-fuel price is varied by $\pm 20\%$ around the base-case value, the simple payback of the hybrid PV–DEG system changes only from about 5.02 to 5.24 years. By comparison, a $\pm 20\%$ variation in electricity price changes the payback from about 5.38 to 4.90 years.

Figures 5 and 6 illustrate the influence of diesel-fuel and electricity price variations on payback, respectively.

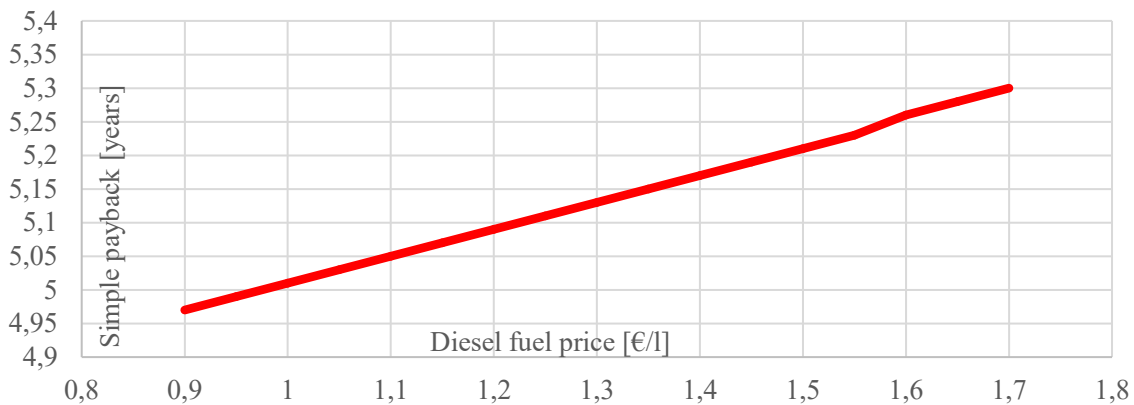


Figure 5. Sensitivity of the hybrid PV–DEG system simple payback to diesel-fuel price variation

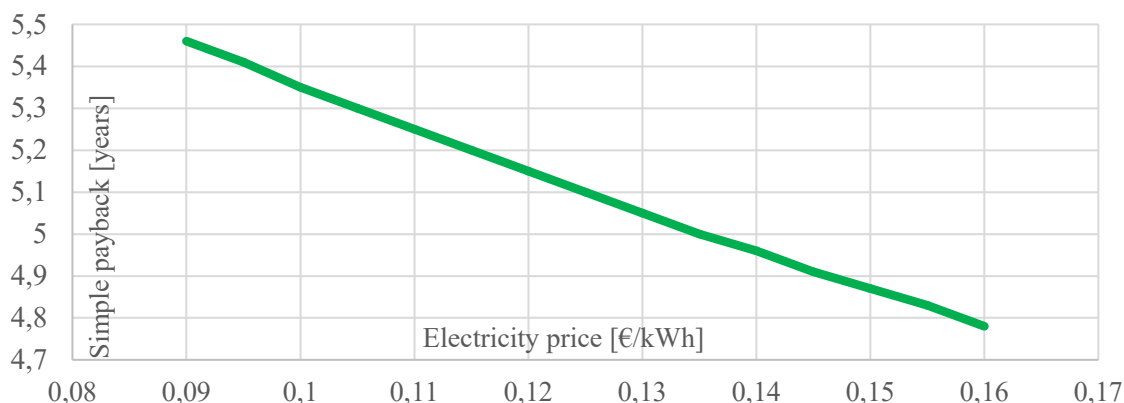


Figure 6. Sensitivity of the hybrid PV-DEG system simple payback to electricity price variation

These results indicate that the hybrid concept is affected by both electricity and fuel prices, but its economic performance remains less sensitive than that of the on-grid concept, since its dominant economic benefit arises from avoided frost-related crop losses rather than from electricity savings alone.

Therefore, the sensitivity analysis confirms two important conclusions: first, the profitability of the on-grid system is strongly driven by the electricity price; and second, the hybrid concept remains economically robust even under moderate increases in diesel-fuel price, because the generator operates only during limited critical periods.

CONCLUSION

Agrisolar systems should be understood not as a single standardized technology, but as a flexible framework for integrating photovoltaic generation with different forms of agricultural production and infrastructure. Their relevance extends beyond renewable electricity generation, since they can contribute to improved land-use efficiency, reduced dependence on external energy supply, better support for irrigation and farm electrification, moderated microclimatic conditions and partial integration with active frost-response strategies. At the same time, their successful implementation requires careful consideration of crop response to shading, structural and operational constraints, maintenance access, and the reliability requirements of agricultural loads. The illustrative 20 ha apple orchard case confirms that the choice of PV supply concept depends primarily on the structure and timing of demand rather than on annual energy consumption alone. The analyzed results show that a 110 kWp on-grid PV system is a technically simple and economically justified solution for reducing annual electricity costs through self-consumption and export of surplus energy. In contrast, a fully autonomous off-grid PV-battery system becomes impractical when dimensioned for rare but large night-time frost-protection loads, due to the excessive required PV and storage capacity. Under such conditions, the hybrid concept combining PV, limited battery storage, and diesel-generator support provides the most balanced solution, because it preserves the benefits of solar energy during normal operation while ensuring reliable supply for critical short-duration peak events.

ACKNOWLEDGEMENTS

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REFERENCES

- Al Mamun, M.A., Dargusch, P., Wadley, D., Zulkarnain N. A., Aziz, A.A. (2022). A review of research on agrivoltaic systems. *Renewable and Sustainable Energy Reviews*, 161, 1364-0321, <https://doi.org/10.1016/j.rser.2022.112351>.
- Barron-Gafford, G.A., Pavao-Zuckerman, M.A., Minor, R.L., Sutter, L.F., Barnett-Moreno, I., Blackett, D.T., Thompson, M., Dimond, K., Gerlak, A.K., Nabhan, G.P., & Macknick, J.E. (2019). Agrivoltaics provide mutual benefits across the food-energy-water nexus in drylands. *Nature Sustainability*, 2(9), 848-855. <https://doi.org/10.1038/s41893-019-0364-5>
- Calero-Lara, M., Lopez-Luque, R., & Casares, F.J. (2021). Methodological Advances in the Design of Photovoltaic Irrigation. *Agronomy*, 11(11), 2313. <https://doi.org/10.3390/agronomy11112313>
- Dupraz, C., Marrou, H., Talbot, G., Dufour, L., Nogier, A., & Ferard, Y. (2011). Combining solar photovoltaic panels and food crops for optimising land use: Towards new agrivoltaic schemes. *Renewable Energy*, 36(10), 2725-2732. <https://doi.org/10.1016/j.renene.2011.03.005>
- Elamri, Y., Cheviron, B., Lopez, J.-M., Dejean, C., & Belaud, G. (2018). Water budget and crop modelling for agrivoltaic systems: Application to irrigated lettuces. *Agricultural Water Management*, 208, 440-453. <https://doi.org/10.1016/j.agwat.2018.07.001>
- Hassanpour Adeh, E., Selker, J.S., & Higgins, C.W. (2018). Remarkable agrivoltaic influence on soil moisture, micrometeorology and water-use efficiency. *PLoS ONE*, 13(11), e0203256. <https://doi.org/10.1371/journal.pone.0203256>
- Liu, T., Zhang, S., Sun, T., Ma, C., & Xue, X. (2025). Review of Active Plant Frost Protection Equipment and Technologies: Current Status, Challenges, and Future Prospects. *Agronomy*, 15(5), 1164. <https://doi.org/10.3390/agronomy15051164>
- Olabode, O.E. et al. (2021). Hybrid power systems for off-grid locations: A comprehensive review of design technologies, applications and future trends. *Scientific African*, 13, e00884. <https://doi.org/10.1016/j.sciaf.2021.e00884>.
- Toledo, C., & Scognamiglio, A. (2021). Agrivoltaic Systems Design and Assessment: A Critical Review, and a Descriptive Model towards a Sustainable Landscape Vision (Three-Dimensional Agrivoltaic Patterns). *Sustainability*, 13(12), 6871. <https://doi.org/10.3390/su13126871>
- Salas, V., Suponthana W., & Salas, R.A. (2015). Overview of the off-grid photovoltaic diesel batteries systems with AC loads. *Applied Energy*, 157, 195-216, <https://doi.org/10.1016/j.apenergy.2015.07.073>.
- Snyder, R.L. (2000). Principles of Frost Protection. University of California.
- Yamegueu, D., Azoumah, Y., Py, X., & Zongo, N. (2011). Experimental study of electricity generation by Solar PV/diesel hybrid systems without battery storage for off-grid areas. *Renewable Energy*, 36, 1780-1787, <https://doi.org/10.1016/j.renene.2010.11.011>.
- Wazed, S.M., Hughes, B.R., O'Connor, D., & Calautit, J.K.S. (2018). A review of sustainable solar irrigation systems for Sub-Saharan Africa. *Renewable and Sustainable Energy Reviews*, 81(Part 1), 1206-1225. <https://doi.org/10.1016/j.rser.2017.08.039>
- Weselek, A., Ehmann, A., Zikeli, S., Lewandowski, I., Schindele, S., & Högy, P. (2019). Agrophotovoltaic systems: applications, challenges, and opportunities. A review. *Agronomy for Sustainable Development*, 39, 35. <https://doi.org/10.1007/s13593-019-0581-3>
- Weselek, A., Bauerle, A., Hartung, J., Zikeli, S., Lewandowski, I., & Högy, P. (2021). Agrivoltaic system impacts on microclimate and yield of different crops within an organic crop rotation in a temperate climate. *Agronomy for Sustainable Development*, 41, 59. <https://doi.org/10.1007/s13593-021-00714-y>

Beekeepers' perceptions of pollination service management in Bosnia and Herzegovina: survey data from the EU COST "Super B" Project and a review of EU and local subsidy policies

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Abstract

Pollination by insects is a key contribution to many crops, with managed honeybees being “hired” to support pollination services in agricultural and natural habitats. The aim of this work was to contribute data to the scientific literature on Bosnia and Herzegovina that addresses an important aspect of local food sustainability through natural but managed pollination services that the European honeybee *Apis mellifera* provides, and to provide insights into existing agricultural subsidies. To our knowledge this is the first pollination management overview based on a standardized approach in Bosnia and Herzegovina. We analyzed results from Europe-wide online questionnaire, administered under COST Action project “Super B”, and compiled data from available sources on pollination service subsidies. The data on beekeeping practices and perceptions of pollination structure were collected using a questionnaire with 178 items grouped into twenty-seven thematic sections and were provided by 109 beekeepers. The study examines beekeeper perceptions and motivations for crop pollination in relation to their demographic characteristics and technological practices. Beekeepers’ perception of mass flowering crops showed a lack of trust in intensive agriculture driven by perceived risks of pesticide contamination and bee mortality. Agri-environment management and subsidy systems at the global and EU level were compared with local policy frameworks, with the intention to promoting more visible and compensated pollination practices in the country. The findings highlight the need for locally adapted and structured recognition of pollination service as well as better options for constructive engagement in pollination services through adequate subsidies. The collected data can serve as a starting point for structuring pollination services and as a step toward improving the pollinator potential of bees, addressing pollination needs, and enhancing local practices in Bosnia and Herzegovina within the broader agricultural pollination service market.

Keywords: questionnaire, pollination, *Apis mellifera*, beekeepers, subsidies

INTRODUCTION

Despite a long history of association with humans, the biology of the western honeybee (*Apis mellifera*) has been explained primarily in recent decades. Advances in omics technologies, molecular genetics, physiology, neurobiology, and behavior have advanced understanding of bee function in the context of pollination biology, while emerging evidence on pesticide exposure, parasites, and pathogens highlights the need for systematic, policy-guided approaches to promote pollination services and optimize their delivery in agricultural ecosystems. The movement of managed colonies increases both honey yields and the value of pollination services, but it also facilitates the spread of pathogens, diseases, and

parasites (Morse & Flottum, 1997). *Apis mellifera* pollination behavior is genetically encoded (gene *Amfor*) and expressed through age-related task allocation within the colony life cycle. By foraging (pollen, nectar) to feed their offspring, these insects transfer pollen among flowering plants, securing plant reproduction, and facilitating gene flow across generations (Terzin, 2017).

Pollinators are a key component of global biodiversity, providing vital ecosystem services to crops and wild plants and pollinator declines can result in loss of pollination services which have important negative ecological and economic impacts that could significantly affect the maintenance of wild plant diversity, wider ecosystem stability, crop production, food security and human welfare (Potts, 2010). Pollination is a key ecosystem service in global crop agriculture. Previous estimates using data from 200 countries found that fruit, vegetable or seed production from 87 of the leading global food crops is dependent upon animal pollination, while 28 crops do not rely upon animal pollination. (Klein, 2007). Insect pollination is critical to round 90% of all flowering plants (Tong, 2023), managed western honeybee *Apis mellifera* is the most abundant and widespread pollinator in the world (Visick, 2023). Managed insects are often key pollinators, particularly in large, homogenous landscapes, with the European honeybee *Apis mellifera* providing approximately half of the recorded crop visits in European studies (Kleijn, 2015) and increase exposure to pesticides, potentially impacting on bee fitness at various scales (Rundlof, 2015; Tsvetkov, 2017).

Trade-offs between benefits (honey yields, pollination fees etc.) and costs vs. risks management costs, low honey quality etc. to beekeepers, can affect decisions on hive placement (Rucker, 2012, Lee, 2018). Although the human food supply is not currently subject to a global pollination crisis, spiraling positive feedback between the impacts of agriculture expansion and pollinator decline on crop yield could accelerate biodiversity loss by promoting further habitat destruction and homogenization (Aizen et al., 2022). Pests, agrochemical toxins, and beekeeping practices can all be sources of colony losses. In Bosnia and Herzegovina, these losses have been less severe than in some other parts of the world: COLOSS questionnaire results from 2008 to 2012 reported annual losses of 10%, 8.6%, 13.7%, and 20.33%, respectively (Santrač, 2022). To improve understanding of beekeeping management and pollination service perceptions, beekeeper responses were analyzed and contextualized within local, regional, and global frameworks to inform future decisions by farmers and policymakers.

MATERIAL AND METHODS

Questionnaire SUPER-B (*Sustainable Pollination in Europe: joint Research on Bees and other pollinators*)

Two separate quantitative surveys were designed, one for beekeepers and the other for growers of insect pollinated crops (hereafter referred to as "farmers"). The questionnaire was approved by the ethics committee of the University of Reading and informed consent was obtained from all participants.

Survey was created and distributed using the online survey platform Qualtrics. All beekeepers' responses were recorded anonymously, identified only by a unique number. Surveys were distributed between September 2015 and March 2016. The survey was translated from English language into the national language and distributed in 21 European or European Near Neighbor countries (Breeze TD, 2019).

The surveys were distributed through established beekeeping channels: existing email contact lists, local beekeeping associations in Republic of Srpska and Federation of Bosnia and Herzegovina, advertised through beekeeping magazines, institutional web pages, and via direct contact with beekeepers during educational events. Collected results from local language were translated back into English by native speaking authors. For BiH question format was prepared in local language on Cyrillic and Latin letters options.

Statistical analyses

Statistical analyses were performed in MicroSoft Excel. A χ^2 test of independence was conducted to assess the association between the type of beekeeper and the number of colonies; between the number of colonies and the need for relocation, as well as between the type of beekeeper and years of experience. Correlation between experience and number of colonies was investigated using Spearman's rank correlation analysis.

Questions with lead answers or descriptive answers were processed with descriptive statistics (mean value and standard deviation).

An insight into the sources of subsidies in the beekeeping sector

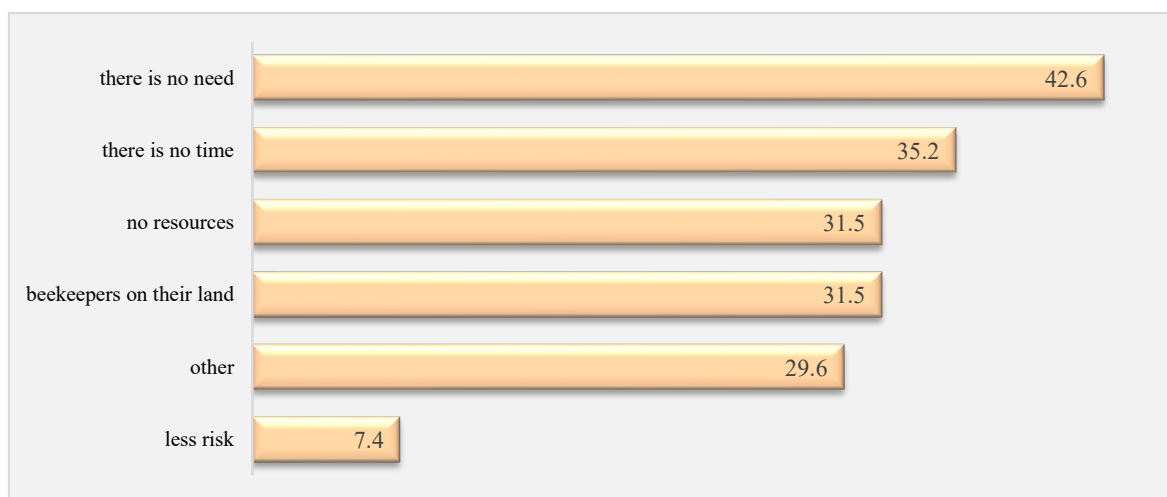
This study employs a literature review combined with document analysis to identify and classify the main sources of subsidies in the beekeeping sector, academic publications, policy reports, and institutional databases were screened and analyzed to provide a comprehensive overview of pollination-related subsidy mechanisms and practices.

RESULTS AND DISCUSSION

Results from Questionnaire

A total of 109 beekeeper responses and only 4 farmer responses were collected. Since the farmer sample did not meet the minimum threshold of 20 responses required for inclusion, BiH was excluded from the final European project data presentation. The low response rate among beekeepers is not atypical. Evidence from previous email surveys suggests that a considerable proportion of beekeepers do not regularly use internet-based communication, which may limit participation in online surveys. In the questionnaire, 24 question parameters were evaluated with a simple way of choosing sometimes multiple answers for a given question. The results in the tables and graphs are given in percentage values. Out of a total of 109 participants, 80.7% (n=88) declared themselves as hobbyist beekeepers and 19.2% (n=21) as professional beekeepers. The respondents managed an average of 47.26 (SD $\pm$ 51.7) hives and kept bees for an average of 13.44 (SD $\pm$ 11.36) years. Professional beekeepers had significantly more years of experience in beekeeping ($\chi^2=11.1$; df = 3; $p < 0.05$) and kept significantly more hives than hobbyist beekeepers. ($\chi^2=33.8$; df = 2; $p < 0.001$). Years of beekeeping experience was positively correlated with hive number ($\rho = 0.714$; $p < 0.001$). Beekeepers with a larger number of colonies move colonies more often ($\chi^2=15.42$; df = 5; $p < 0.05$).

96 survey participants answered the question whether they are moving their colonies. 43.75% (n 42) declared that they practice migratory beekeeping, while 56.25% (n 54) do not. The percentage answers to the question why they don't move are shown in Graph. 1. The "other" option includes: they don't have enough hives, they don't have the means to move, security reasons, bad market, as well as a beginner beekeeper.



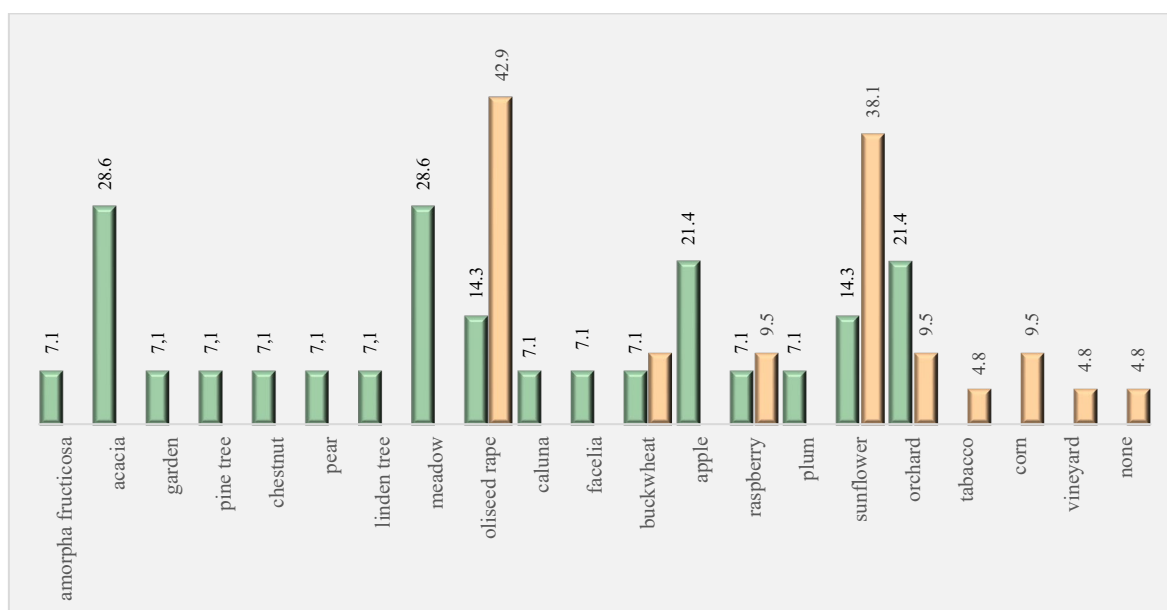
Graph. 1. Reasons for stationary beekeeping

The selection of the natural environment to which the hives are moved is shown in Table 1.

Table. 1. Natural environments to which beekeepers move in the annual seasons

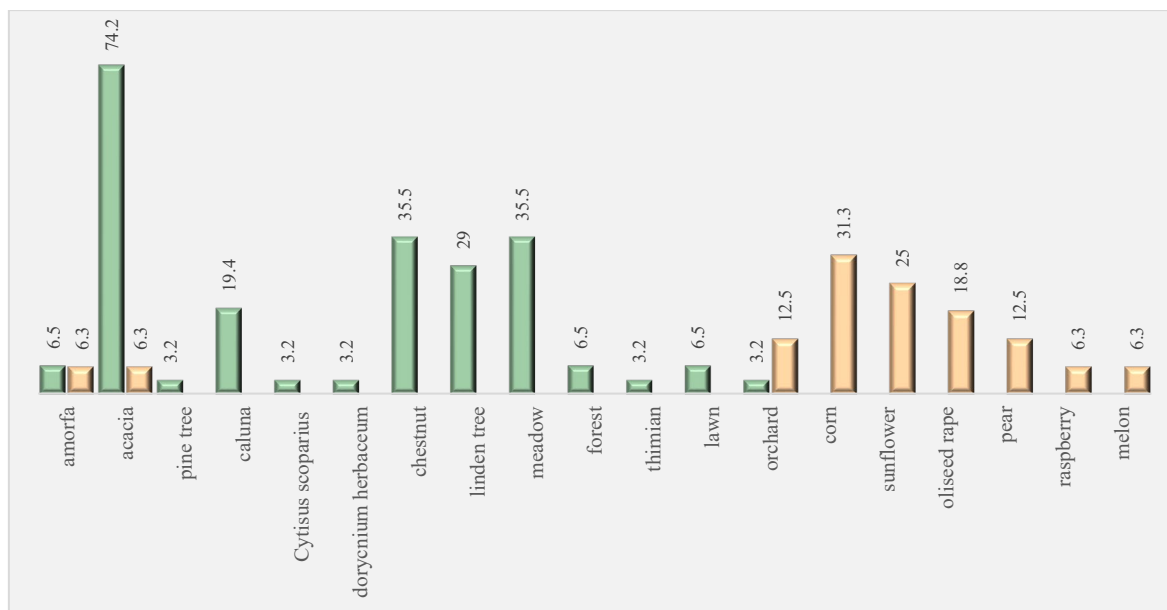
Environment	Spring	Summer	Autumn	Winter
Forest	28	39.4	26.1	20
Agricultural crops	36	9.1	8.7	13.3
Lawn	16	33.3	43.5	33
Meadow	8	21.2	17.4	13.3
Garden	-	3	4.3	2
Pasture	4	-	-	-
Orchard	4	-	-	-
Suburbs	-	-	-	6.7
Acacia	12	-	-	-

Most beekeepers rank forest and lawn highly, regardless of the season, followed by agricultural crops and meadows. The garden as an option was chosen the most in winter, while beekeepers indicated pasture and orchard only in spring, as well as suburban only in winter. Graph. 2. Presents the percentage of responses identifying desirable and avoided crops near hives. Green bars are desirable and yellow bars are avoided.



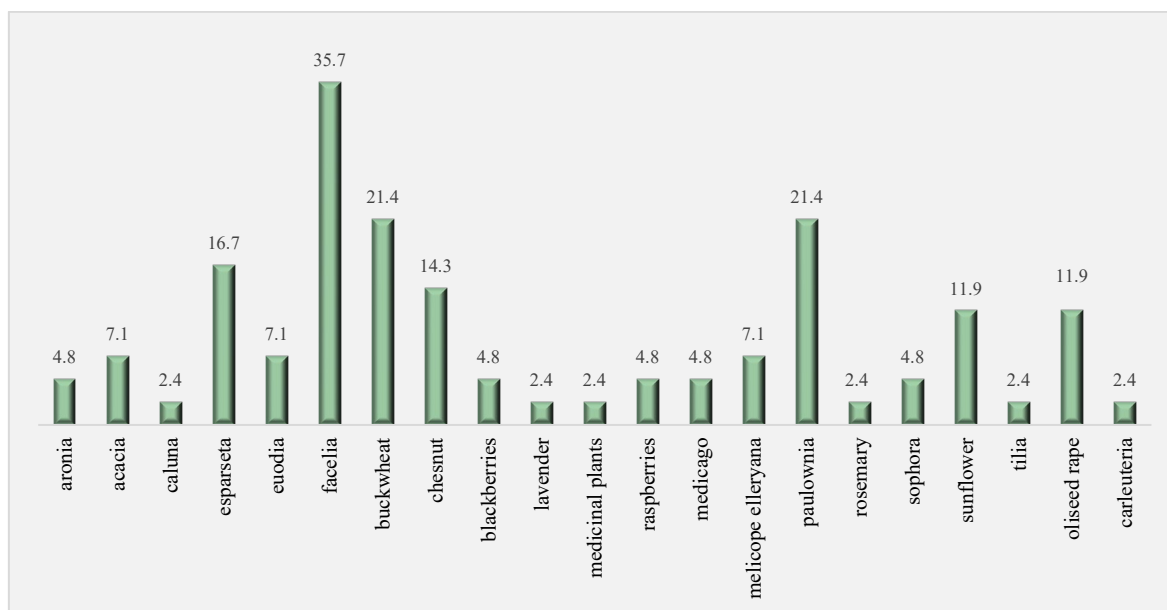
Graph. 2. Agricultural crops that beekeepers prefer and avoid

Beekeepers who move their colonies prefer acacia and meadow, followed by apple, orchard, oilseed rape, and sunflower. Of the crops they avoid, rapeseed, sunflower, followed by corn, orchard and buckwheat were chosen the most. It is interesting that there are agricultural crops that a certain percentage of beekeepers choose and avoid (canola, sunflower, buckwheat, raspberry, and orchard). Oilseed rape and sunflower were avoided crops, while for the same crops beekeepers reported in smaller numbers that they preferred those crops. Graph. 3. shows the percentage values of the answers to the questions which plants prefer and avoid near their hives. Green bars are desirable and yellow bars are avoided.



Graph. 3. Plants that beekeepers prefer or avoid

And when it comes to the question of which plants most often move to, beekeepers favor acacia, followed by chestnut and meadow, and caluna is preferred. Among the plants they avoid are corn, sunflower and rapeseed. Amorphous is classified as desirable and undesirable in an approximate percentage, while more beekeepers choose it as undesirable for the orchard. Graph. 4. shows the percentage of responses that they want a plant near their hives.



Graph. 4. Plants that beekeepers would like near their hive

For the most desirable plants, beekeepers list phacelia, followed by buckwheat and paulownia, followed by esparseta and chestnut. All the listed plant species deserve to be included in future honey plant planting plans because they are recognized by beekeepers in our geographical area. Improvement of capacity for planting and seed material of these species should be encouraged institutionally. Table 2. lists the reasons why beekeepers move or do not move to agricultural crops or plants. Survey participants could choose several answers to these questions. Percentages exceed 100% because respondents could select multiple options. The results represent the percentage values of the answers.

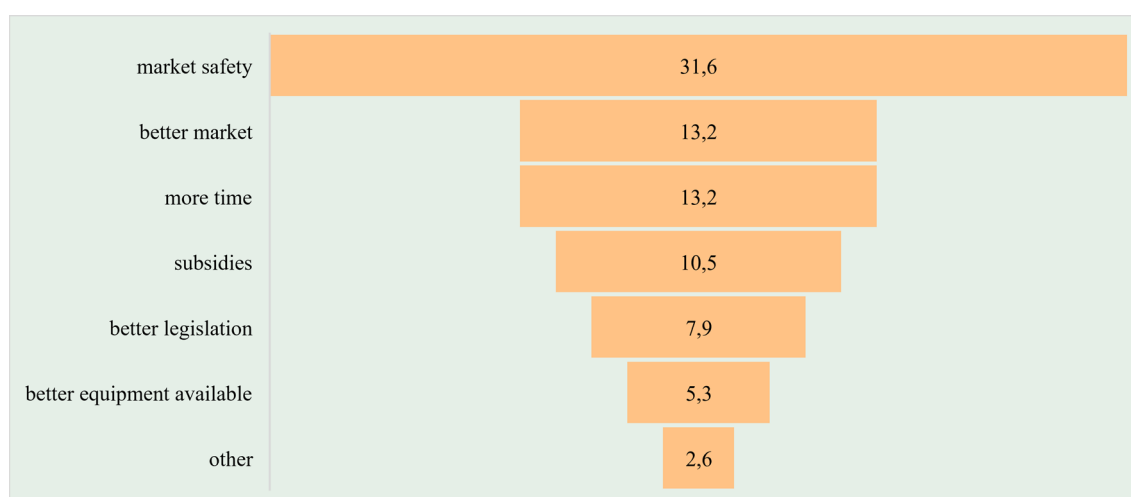
Table 2. Criteria for preferring or avoiding agricultural habitat and natural habitat

Prefer			Avoid		
Criterion	Agricultural habitat	Natural habitat	Criterion	Agricultural habitat	Natural habitat
Yields	119.5	84.8	Yields	67.7	86.7
Availability	75.6	21.2	Quality	19.4	46.7
Sustainability	63.4	57.6	Other sources	22.6	53.3
The owner	56.1	21.2	Contains poisons	38.7	40
Other	41.5	60.6	Pesticide risk	6.5	
Quality	14.6	84.8	Not paid	16.1	
Recommendation	14.6	63.6	Advised	51.6	73.3
Development	9.8	36.4	Risk of disease	29	13.3
Asked - paid	9.8	63.6	Requested	77.4	6.7

Honey yield was chosen by most beekeepers as the reason for relocation, regardless of whether they are moving to agricultural crops or natural habitat. The quality of honey, availability (proximity), recommendation, significance for the sustainability of hives, as well as asked-paid answers (n=4) were also highly ranked. In the "other" category, they mark good and reliable yields, as well as high-value honey.

Why do they avoid certain agricultural crops? The highest percentage of answers was that the owner of the crops asked them not to move to his crops. Insufficient honey yields are a highly ranked reason for avoiding some plants as well as agricultural crops. In some responses beekeepers were advised not to move hives to certain plants, and other sources, which were common reasons for not moving. Risk of disease was recognized but not enough as real risk for bees.

As factors that can contribute to the increase in the number of beehives, market security, a better market and more time, subsidies, and better legislation stand out. The percentage values of the answers are shown in Graph. 5.



Graph. 5. Key factors for increasing hive numbers

In the „other” category, beekeepers state: better equipment available, better accessibility of sites, better management of the apiary, beekeeping as the only job, workforce, age restrictions, higher yields, higher profit, more food available and *paid pollination services*. The survey participants also mentioned factors

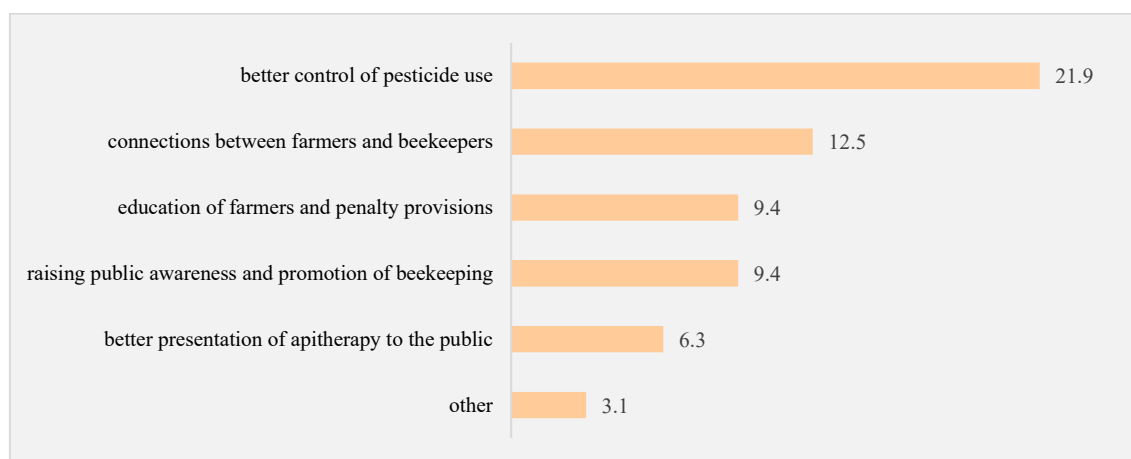
with which both farmers and regulatory authorities would contribute to greater engagement in pollination. The percentage value of the answer is shown in Table 3.

Table 3. Beekeepers' perspectives on measures that could boost greater participation in pollination services

Regulatory authorities	Farmers
➤ 54.8% control of the use of pesticides ➤ 18.2% better legislation ➤ 9.1% subsidies for beekeeping ➤ 3% better marker, better extension services, registration control, beekeeper-farmer lobbying, better beekeeping practices, more education	➤ 29.7% control of the use of pesticides ➤ 27% better beekeeper-farmer relationships ➤ 21.6% paid pollination service ➤ 8.1% crops more suitable for bees ➤ 5.4% better education, organic production ➤ 2.7% free access to the crop, better market

The statements given in the table in relation to regulatory bodies and farmers, i.e. growers of plant crops, clearly recognize the current conflict zones, i.e. the need to improve ecological agricultural practices on the territory of Bosnia and Herzegovina, high-ranked recognition of the burden of pesticide contamination the need for better communication between beekeepers and farmers, then the need for clearer legislation, then subsidies in the field of beekeeping and paid pollination services, adjustment of conditions for the honey flora, also education and organic production and permitted approaches to agricultural crops are cited by a significant percentage as measures to improve the pollination service in Bosnia and Herzegovina.

Comparable results were achieved in EU scale questioner (Breeze TD, 2019). Survey participants were able to list proposals related to beekeeping and improving pollination. The percentage results of the responses are shown in Graph. 6.



Graph. 6. Proposals by beekeepers for enhancing pollination services

In the „other” category, beekeepers state: better quality control of bee products, better legislation, education about the importance of pollination, sustainable production, promoting the importance of pollination services, paid pollination system, subsidies for beekeeping, media influence for better communication, greater responsibility, more honey plant species, more feed sources available for free, the importance of pollination for local colonies. Pesticides remain an unresolved issue, as are the links between farmers and beekeepers. They believe that education, presentation, and promotions, raising public awareness can lead to improved pollination.

Subsidies practices sources data findings

Agricultural pollination services are an issue that will gain even more importance in the near future with all the necessary elements and options for improvement. Pollination is not only related to honeybees, but also to bumblebees, orchard bees, and other wild pollinator species of an ecological system directly related to the demands of organic agricultural practices with the increase and increased emphasis on sustainable agriculture, so called natural production. Orchard pollination focuses on orchards, where bees improve crop yields and quality. Pollination in agriculture encompasses a wider range of crops, including vegetables and cereals, which benefit from a variety of pollinator services. The “other” category includes niche markets such as greenhouse pollination or specialty crops. In North America, especially in the United States and Canada, the market for agricultural pollination services is strong. The US is a major user of commercial pollination services, particularly for high-value crops such as almonds, blueberries and apples (Morse & Flottum, 1997). Recent market trends indicate a shift towards integrated approaches to pollination, including the adoption of managed pollinator species and habitat protection strategies.

EU targeted ‘environmental, climate-related and other management commitments (AECM)’ Rural Development interventions supporting beekeeping– (as in BG, EE, ES, FR, HU, IT, LT, LV, MT, PT) (e.g. BG II.A.12 *Promoting natural pollination*; IT ACA18 *Commitments for Beekeeping*; LV LA10.4 *Beekeeping units for pollination needs*; FR 70.29 *Improving pollinator potential of bees*; ES 650.14 *Beekeeping for biodiversity*). Other AECM creation maintaining biodiversity cover, planting of melliferous plants, Organic farming, Integrated pest management pesticide bans etc. Contributing eco-schemes: most involve maintaining creating habitat for pollinator species and or increasing the availability of food sources for pollinators in agricultural landscapes (e.g. buffer strips, planting of melliferous plants, extensive grassland management, ecological focus areas.

While agricultural markets in the European Union and United States are highly subsidized and apply production function approaches to value pollination, such recognition is lacking in Bosnia and Herzegovina, indicating the need for structured research and policy integration to establish pollination services as a recognized component of beekeeping activity. Support to beekeepers from the agrarian budget at the level of BiH is implemented once a year on the principle of direct payments for bee colonies, through associations or cooperatives. Also, beekeepers get this kind of support per hive from the budgets of local colonies and entities. From the entity budget and the Federal Ministry of Agriculture, Water Management and Forestry, (SNFBIH 33/24), Republic of Srpska (SGRS 16/26) investments in capital investments for the purchase of equipment and the construction of buildings on bee farms are also subsidized. Due to the importance of bees for agriculture and the environment, monetary incentives are also provided as part of systemic measures, such as support for breeding and selection activities in beekeeping, which indirectly contributes to the preservation of locally selected queens that are important for the geographical area and adapted to the pollination of plant species. *Bee breeding program in the RS for the period 2025-2027*. Within this Program, there are also subsidies for the establishment and operation of test stations, as well as support for institutions for monitoring and controlling the success of selection. Bosnia and Herzegovina participates in broader Western Balkans agricultural policy where beekeepers are supported by subsidies per hive and by rural development measures for investments, certification, organic production, processing, and marketing. Preparation for IPARD-like support, EU pre-accession funds for agriculture and rural development, includes potential measures where pollinators and beekeeping fit under environmental, biodiversity, and climate-resilience axes. These are more strategic medium term, but keeping your project ideas aligned with IPARD-type priorities (environmental services, biodiversity, rural income) will help later.

Donor, UN, and NGO programs in BiH have used beekeeping to support vulnerable groups (e.g. mine victims UDAS) with income generation and rehabilitation; these are typically supported by international donors, UN agencies, or NGOs, and can fund hives, equipment, and training. International pollinator initiatives BES-Net, Promote Pollinators work on pollinators in BiH highlight the lack of monitoring and the need for better policies and funding for pollinators, which creates an entry point for research and pilot projects with external financing.

Rising concerns over food security and declining pollinator populations are accelerating the recognition of agricultural pollination as a critical service extending beyond honeybees to other managed species within

increasingly integrated and sustainability-driven systems, a trend already well established in markets such as the United States and Canada (Morse & Flottum, 1997) In Europe, key markets such as Germany, France, the U.K., Italy, and Russia are influential. Germany and France are proactive in promoting biodiversity and sustainable farming, leading to a growing reliance on pollination services. The U.K. has seen an increase in organic farming, which often depends on pollinators. Italy is known for its vineyards and fruit orchards, while Russia, with its vast agricultural land, is beginning to emphasize the importance of pollination in improving yield. Agricultural Pollination Service Market is witnessing significant growth across regions driven by the increasing awareness of the role of pollinators in food production, alongside changing agricultural practices and the need for sustainable farming solutions.

The FAO statistical database (Sorrenti et al., 2020) and estimate that FAOSTAT reports Bosnia and Herzegovina's honey production at 3.992 tons in 2018, based on official national data, positioning it among smaller European producers amid a global total of about 1.870 thousand tons that year. Recent secondary sources citing FAO trends indicate 350.000–446.000 bee colonies, with 2023 honey output around 4.280 tons (down 9.3% year-over-year but up 59% over three years), reflecting growth from earlier figures despite climate and disease pressures.

Economic Valuation of Pollination Services: Review of Methods, produced for the FAO coordinated focus on “Conversion and management of pollinators for sustainable agriculture, through an ecosystem approach, June 2006, state this: *“Nevertheless, sometimes conflicting value estimates for the pollination service have been produced and the available information is prone to considerable uncertainty”* (FAO 2006). Agricultural markets in the EU and the US are characterized by large-scale subsidies. As these subsidies increase farmers’ income per unit of agricultural products produced, it can be expected that, in general, these subsidies lead to an upward bias in the value estimate, i.e. the value is likely to be lower than economic value of the pollination service at the national scale can only be assessed with production function approaches.

No peer reviewed paper to have calculated a monetary economic value of pollination services specifically for Bosnia and Herzegovina; existing literature notes the importance of pollinators and honey production without a formal valuation. Support to beekeepers from the agrarian budget at the level of BiH is implemented once a year on the principle of direct payments for bee colonies, through associations or cooperatives. Also, beekeepers receive this type of support from the budgets of local colonies and entities. From the budget of the entity and the federal Ministry of Agriculture, Water Management and Forestry, investments in capital investments are subsidized for the purchase of equipment and the construction of buildings on bee farms.

Due to the importance of bees for agriculture and the environment, monetary incentives were also provided as part of systemic measures, support for breeding and selection activities in beekeeping, which indirectly contributes to the preservation of selected queens that are important for the geographical area and adapted to pollination of plant species.

The importance of bees as pollinators is indirectly supported at the same time through breeding and selection of the most resistant species and subsidies for the establishment and operation of test stations as well as support for monitoring and control institutions. Within the bee breeding program in the RS for the period 2025-2027 year, activities are planned that will enable better availability of information to the widest possible circle of beekeepers and introduce the public to the importance of bees in nature. in connection with this, the importance of beekeeping seen through the production of bee products is far less compared to their role in pollination, which makes beekeeping even more important. Within the support preparation activities, such as the IPARD program, potential measures where pollination and beekeeping fit into the environmental and biodiversity protection program are included. However, the International Pollination System Initiatives BES - Net / Promote Pollinators point out the lack of monitoring and the need for a better incentive policy and financing of pollination services. Using Questioner data as a tool for gain answers that was not present in larger scale for beekeeping management practice and pollination practice in connection farmers (plant growers) in the one we did not meet all criteria that all what was planned we reached. The low number of farmer responses (4) was insufficient to assess their pollination perceptions, needs, and preferences, which could otherwise be utilized through synergistic practices for more efficient pollination.

In Bosnia and Herzegovina there are no compensations for pollination services to hives that can be nomadic to locations with agricultural plant plots. In the United States, for example, the large-scale demand for almond pollination in 2026 commanded approximately \$200 per hive during the February–March pollination season (Seth Rick, personal communication, March 2026). Based on data from the National Agricultural Statistics Service (NASS), pollination prices in regions 6 & 7 rose in 2025, with almond colony costs increasing from \$181 to \$209 (+15%) and per-acre prices rising slightly from \$305 to \$310. The total value of almond pollination grew by 5%, contributing to an overall pollination market value of \$364 million in 2025 (+3% from 2024), with almonds remaining the highest-valued crop in the region. (USDA NAS, 2026,). After the pollination they reach apples and other cultures like melons, pumpkins etc. were also paid. For some huge beekeeping operations that practice is main profit income. Because we are in Europe, not in EU, but with perspective to harmonize their legislative frameworks in near future we evaluate subsidized practice in BiH, and with FBiH and RS levels. Within the 2023–2027 Common Agricultural Policy, the EU supports beekeeping through mandatory apiculture interventions and agri-environmental measures, which aim to sustain honeybee populations and their pollination services, although these services are not yet remunerated as a distinct ecosystem service,

CAP Strategic Plans (2023-2027) Summary overview for twenty-seven member states is only a few member states explicitly label beekeeping interventions as targeting pollination (not just honey production), and these are framed as environmental public good measures rather than per-hive “pollination payments”.

CONCLUSION

Although based on a limited and potentially biased sample, this survey provides indicative insights into beekeeper perceptions and identifies key knowledge gaps, highlighting the need for more extensive and representative assessments. It establishes initial evidence base on the status and understanding of pollination in Bosnia and Herzegovina, offering a foundation for future research and policy development. The findings emphasize that pollination services remain under-recognized despite their substantial ecological and economic importance, and they point to the necessity of integrating pollination more clearly into agricultural support frameworks. Given the findings, the legislative framework should be strengthened by incorporating input from agricultural producers who rely on pollination services. This is particularly important as beekeepers identified inadequate pesticide regulation and associated poisoning risks to honeybees as a key concern. The first step can be organized educational system for beekeepers and agricultural producers concerning the pollination issues. Strengthening knowledge transfer mechanisms is essential to ensure that scientific evidence more effectively reaches policymakers, practitioners, and the wider public, thereby enabling informed decision-making and the development of sustainable pollination management systems. We recommend that Bosnia and Herzegovina adopt a paid pollination scheme modeled on European Union Agri-Environment-Climate Measures (AECM) to strengthen pollination services, support beekeepers, and enhance agricultural sustainability.

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REFERENCES

- Aizen, M. A., Garibaldi, L. A., & Harder, L. D. (2022). Myth and reality of global crisis for agricultural pollination. *Ecología Austral*, 32, 698–715. <https://doi.org/10.25260/EA.22.32.2.1.1875>
- Breeze, T.D., Boreux, V., Cole, L., Dicks, L., Klein, A. M., Pufal, G., Balzan, M. V., Bevk, D., Bortolotti, L., Petanidou, T., Mand, M., Pinto, M. A., Scheper, J., Stanisavljević, L., Stavrinides, M. C., Tscheulin, T., Varnava, A., & Kleijn, D. (2019). Linking farmer and beekeeper preferences

- with ecological knowledge to improve crop pollination. *People and Nature*, 1, 562–572. <https://doi.org/10.1002/pan3.10055>
- FAO 2006: Tools for Conservation and Use of Pollination Services, Economic Valuation of Pollination Services, <https://www.fao.org/fileadmin/templates/agphome/documents/Biodiversity-pollination/econvaluepoll1.pdf>
- General Information on Apiculture Interventions in CAP Strategic Plans (CSP's) (2023-2027) https://agriculture.ec.europa.eu/document/download/d56e99be-bc25-47ab-88c4-a2cf9b0ea586_en?filename=overview-apiculture-interventions-csp-may-2024_en.pdf&prefLang=sl
- Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L. G., Henry, M., Isaacs, R., & Potts, S. G. (2015). Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nature Communications*, 6, 7414. <https://doi.org/10.1038/ncomms8414>
- Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B – Biological Sciences*, 274(), 1608, 303–313. <https://doi.org/10.1098/rspb.2006.3721>
- Lee, H., Sumner, D. A., & Champetier, A. (2018). Pollination markets and the coupled futures of almonds and honey bees: simulating impacts of shifts in demands and costs. *American Journal of Agricultural Economics*, 10, 230–249. <https://doi.org/10.1093/ajae/aay063>
- Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Pollinator declines: Trends, impacts and drivers. *Trends in Ecology & Evolution*, 25, 345–353. <https://doi.org/10.1016/j.tree.2010.01.007>
- Morse, R. A., & Flottum, K. (Eds.). (1997). *Honey bee pests, predators, and diseases* (3rd ed.). Root Publishing., ISBN 0-936028-10-6
- Rucker, R. R., Thruman, W. H., & Burgett, M. (2012). Honeybee pollination markets and the internalization of reciprocal benefits. *American Journal of Agricultural Economics*, 94, 956–977. <https://doi.org/10.1093/ajae/aas031>
- Rundlof, M., Andersson, G. K. S., Bommarco, R., et al. (2015). Seed coating with a neonicotinoid insecticide negatively affects wild bees. *Nature*, 521, 77–80. <https://doi.org/10.1038/nature14420>
- Santrač, V. (2022). Biological and economic significance of beekeeping. In: Trkulja R., Grujic R., Przulj N. (eds) Nutritional and economic importance of livestock. Academy of Sciences and Arts of the Republic of Srpska, Banja Luka, Monograph XLVII: 445-489 Vol. 4 No. 4 (2022): Nutritional and economic importance of livestock
- Sorrenti, S., Muir, G. & Jannoni, R. (2020). *Honey & Beeswax data profile*. Rome. FAO (2020) <https://www.fao.org/forestry/nwfp/statistics/honey-and-beeswax/en>
- Tong, Z. Y., Wu, L. Y., Feng, H. H., Zhang, M., Armbruster, W. S., Renner, S. S., & Huang, S. Q. (2023). New calculations indicate that 90% of flowering plant species are animal pollinated. *National Science Review*, 10, 10, nwad219. <https://doi.org/10.1093/nsr/nwad219>
- Terzin, T. (2017). Medonosna pčela jevanđelje prirode, drugo izdanje, Beograd, Preporod. ISBN 978-86-423-0505-9, str. 262.
- Tsvetkov, N., Samson-Robert, O., Sood, K., Patel, H. S., Malena, D. A., Gajiwala, P. H., Zayed, A. (2017). Chronic exposure to neonicotinoids reduces honeybee health near corn crops. *Science*, 356, 1395– 1397. <https://doi.org/10.1126/science.aam7470>
- USDA NASS. (2026). Cost of Pollination. United States Department of Agriculture, National Agricultural Statistics Service. <https://esmis.nal.usda.gov/sites/default/release-files/795775/cospol25.pdf>
- Visick, O. D. & Ratnieks, F. L. W. (2023). Density of wild honey bee, *Apis mellifera*, colonies worldwide. *Ecology and Evolution*, 13, e10609. <https://doi.org/10.1002/ece3.10609>

- Правилник о условима и начину остваривања новчаних подстицаја за развој пољопривреде и села (SGRS 16/26) <https://vladars.rs/sr-SP-Cyrl/Vlada/Ministarstva/mps/media/vijesti/Documents/Pravilnik%202026.pdf>
- Pravilnik o uslovima i načinu ostvarenja novčanih podrški po modelu ruralnog razvoja (SNFBIH33/24) <https://fimpvs.gov.ba/wp-content/uploads/2024/06/03-PRAVILNIK%20-%20poticaj%20proizvodnje%20-%2043%202024.pdf>
- Program uzgoja pčela u Republici Srpskoj za period 2025-2027.- *Bee breeding program in the RS for the period 2025-2027* <https://vladars.rs/sr-SP-Cyrl/Vlada/Ministarstva/mps/PPP/Pages/Splash.aspx>
- BES Net Promote Pollinators work on pollinators in BiH https://www.besnet.world/wp-content/uploads/2016/12/event-document_Vesnic_Adi.pdf
- INSTRUMENT FOR PRE-ACCESSION ASSISTANCE (IPA II)2014-2020, EU Support to Agriculture and Rural Development, Food Safety, Veterinary and Phytosanitary Standards [ipa_2018_41501_ad10_bih_eu_support_to_agriculture_and_rural_development_food_safety_veterinary_and_phytosanitary_standards_.pdf](https://www.besnet.world/wp-content/uploads/2016/12/event-document_Vesnic_Adi.pdf)
- Pčelarstvo kao alat u osnaživanju žrtava mina u BiH, <https://www.udas.rs.ba/la/pcelarstvo-kao-alat-u-osnazivanju-zrtava-mina-u-bih-2/>
- BES net Bosnia and Herzegovina , https://promotepollinators.org/wp-content/uploads/sites/507/2021/01/Handout_Ena_Hatibovic_Bosnia_Herzegovina.pdf - Search

Color and antioxidant activity in light colored traditional autochthonous apple varieties

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Abstract

As a part of the Gene Bank of the Republic of Srpska, within the Institute of Genetic Resources, University of Banja Luka, field collections of autochthonous fruit and grape varieties serve as a valuable resource for diverse scientific research, experimental studies, measurements, and long-term monitoring. In ripening season of 2024, we conducted research on traditional autochthonous apple varieties. From field collections, we selected 3 apple accessions with good yield potential, for sampling. The apple varieties included in this study were Bjeličnik, Vidovka žuta and Kanada Švabica. After color analyses was done, we conducted analyses of antioxidant potential in samples. Methods used for color were the LAB digital color positioning system and for antioxidant potential, it was determined using the DPPH free radical sequencing method. Results showed that EC₅₀ value of 18.60 mg/mL places 'Vidovka žuta' at the top of the studied varieties for antioxidant potential. 'Bjeličnik' is interesting variety in the pomology of Republika Srpska, named for its characteristic pale, almost white skin (EC₅₀ = 24.19 mg/mL). Kanada švabica showed EC₅₀ of 31.87 mg/mL, and suggests a lower relative antioxidant potency compared to 'Vidovka žuta' and 'Bjeličnik'. Color showed that Bjeličnik is also significantly lighter than the others, confirming its phenotype as the "white" apple of the collection. Yellowness (b*) indicated that Vidovka žuta and Kanada švabica are statistically similar in their yellow intensity, while Bjeličnik is significantly less yellow. Traditional apples are a rich source of dietary antioxidants. The high uniformity in 'Bjeličnik' and the superior potency of 'Vidovka žuta' justify their use in breeding programs targeting increased functional value in modern apple cultivars.

Keywords: apples, color, antioxidant potential

INTRODUCTION

Plant genetic resources require permanent conservation, maintenance and reproduction in order to ensure diversity in food supply and to provide access to producers, breeders, and researchers. Genetic resources of autochthonous fruit trees are an important resource for the protection of biological diversity, sustainable development, and preservation of ecosystems. The value and importance of old traditional fruit varieties are reflected in their diversity, resistance to diseases and pests, nutritional values, and therefore the content of biochemical components that indicate the antioxidant potential of the observed fruit species. This gene pool represents the big potential for studying the pomological and biochemical characteristics of a large number of traditional autochthonous apple and pear varieties.

Nutritional value of domestic apples can be a good basis for a healthy diet for people and a medical recommendation for the prevention of many diseases. Apples and pears are popular among consumers because of their pleasant taste and high content of bioactive, health promoting phytochemicals. Apples represents the most important varieties of pome fruits grown in moderate climate zone. Pomological and

organoleptic properties of apple fruits are highly valued by consumers. In addition to fresh consumption, apple fruits are used as raw materials for different types of processing, such as purees, juices, jams, dried and so on (Kolniak - Ostek, 2016). However, changes in ecological factors due to global climate change make production less sustainable and economical (Gul et al., 2014). It is reported that the burns caused by light and temperature in late varieties, lead to serious marketing and storage problems. It was also reported that the struggle against the *Cydia pomonella* and *Venturia inequalis*, epidemics of which peaked during this period was ineffective and residue problems were experienced due to intense (about 20 times in the season) chemical management. These situations increase the production cost while reducing the market value (Mertoglu et al., 2022).

Biochemical composition of apples is affected by genetic and environmental factors and, therefore, differences between varieties of the same species and even between different orchard environments. Biochemical characterization and analyzing of antioxidant activity are useful tools to identify varieties with superior organoleptic properties and potential health benefits (Commisso et al., 2021). Being high in fiber, low in calorie, and rich in vitamins and minerals, apples is a nutrient-dense fruit. Contents of free sugars and organic acids play an important role in the nutritive value and quality of fruit, due to the formation of sour taste and regulation of chemical reactions in the body (Kolniak-Ostek 2016).

Apples are the most consumed fruit, and they contain various bioactive compounds, including pectins, dietary fibers, vitamins, oligosaccharides, triterpenic acids, and phenolic compounds such as flavonols, dihydrochalcones, anthocyanidins, hydroxycinnamic acids, and hydroxybenzoic acids. Because of the higher content of these phenolic compounds, apples are well known for their high antioxidant potential. The purpose of using antioxidants in a food product is to delay the oxidation reaction by inhibiting formation of free radicals or by interrupting the propagation of free radicals (Arias et al., 2022). In general, many plant tissues develop antioxidant compounds under constant oxidative stress to protect themselves from free radicals, reactive oxygen species, and pro-oxidants generated both exogenously (heat and light) and endogenously (H_2O_2 and transition metals). These antioxidant species are known as natural antioxidants. Some common examples of natural antioxidants are flavonoids, phenolic acids, carotenoids, and vitamins (ascorbic acid, beta-carotene, tocopherols). They are efficient in scavenging free radicals, chelating pro-oxidant metal ions, or acting as reducing agents (Asma et al., 2023).

Patocka et al. (2020) said that apples are rich in various polyphenols: apples accumulate hydroxycinnamic acids (chlorogenic acid), flavan-3-ols, procyanidins, flavonols (quercetin), dihydrochalcones (phloretin) and (in red-peel varieties) anthocyanins. As recently reviewed, these metabolites exert antioxidant activity both on human after fruit consumption and on fruits, that accumulate them in order to keep their REDOX homeostasis.

Apples have a growing scientific interest because many investigations have demonstrated their beneficial effects on human health. Through a variety of antioxidant ingredients, apples have been associated with reduced risks of degenerative and cardiovascular diseases, which are considered to be caused by oxidative stress, especially by free radicals and reactive oxygen species (Hyson, 2011). In apples polyphenols are the most abundant antioxidants in the human diet and the major part of antioxidants, rather than essential nutrients such as vitamin C. Apples are one of the most important fruit sources of dietary polyphenolic compounds in the Western diet, due to the fact that they are consumed widely and are available throughout the year. In Germany, they are the most popular type of fruit with an annual consumption of 25.9 kg per person. Various reports indicate that the polyphenolic profile and content as well as the antioxidant capacity in apples are affected by different variables, such as variety, tissue zones, harvest time, geographic location and storage conditions (Wolfe et al., 2003).

In recent decades, new apple cultivars, such as Braeburn, Elstar, Golden Delicious, Granny Smith and Jonagold have become more popular among consumers in Germany and Western Europe, resulting in a gradual decrease of growing old varieties. New varieties are known to have a lower content of polyphenols. The contents of polyphenolic compounds of new varieties were reduced by breeding due to the astringent taste and rapid enzymatic browning. Therefore, we hypothesize that the content of polyphenols of old traditional apple varieties are higher compared to new varieties (Kschonsek et al., 2018).

Polyphenols are important plant-derived secondary metabolites, which include hydrolyzed tannin (acid ester polyphenols) and condensed tannin (flavanols polyphenols or proanthocyanins) (Katz et al., 2020).

Polyphenols consist of aromatic rings with attached hydroxyl groups, organic acids and acylated sugars. This unique structure endows polyphenols with high antioxidant activity which can directly or indirectly prevent the formation of free radicals. The most abundant polyphenols in pears include flavan-3-ols, flavonols, phenolic acids, anthocyanins and hydroquinones. The antioxidant potential of polyphenols in fruits can be determined by several chemical assays each of which depend upon different mechanisms. These include 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay (Wang et al., 2021).

Our goal in this research was to evaluate color and antioxidant activity in 3 traditional autochthonous light colored apple varieties. Our main goal was to determine values related to color and antioxidant potential and differences in color and antioxidant value between individual varieties of apples.

MATERIALS AND METHODS

As a result of many years of collection and multiplication, as a part of Gene Bank of Republic of Srpska, In the Institute of Genetic Resources, in 2013, we established a Field collection of autochthonous fruit and grape varieties. Entire collection covers an area of 1.2 acres and consists of a large number of various autochthonous accessions of apples, pears, plums, cherries. More than 450 accessions were introduced into the field collection, more precisely into the gene bank.

In these collections, trees are grown and maintained in local ecological and orographic conditions and represent a large pool of various types of scientific research, experimental setups, measurements, monitoring. We conducted one of such studies, in the fruit ripening season of 2024. It is important to note that autochthonous apple varieties are highly resistant to diseases, pests and adverse climatic conditions, and accordingly, no chemical treatments or treatments to improve the qualitative attributes of the fruits were not performed in field collections. These trees live in natural local conditions with minimum of our influence.

From field collection of traditional autochthonous apples, we selected a total of 3 accessions that had good yield potential from which representative samples could be taken. The following apple varieties were included in this research: Bjeličnik, Vidovka Žuta and Kanada Švabica.



Fig. 1 Sample of variety Vidovka Žuta during analyses



Fig. 2 Apple varieties Kanada Švabica during analyses

Samples from the trees were taken successively according to the time of ripening, and were transferred to laboratory for performance analyses of color and antioxidant potential. A sample from one accession consisted of 10 fruits. Color was measured with colorimeter type X-Rite i1 Basic Pro3 (Switzerland). The device works by placing its sensor on surface of fruit, whose color is being determined, pressing the trigger that activates the flash – light and converting the signal into a "Lab" value. The reading is taken on a display connected to the sensor.

"Lab" digital color positioning system represents three indicators: The "L" indicator indicates the amount of light in the color, and its limit values are black and white. The "a" indicator represents the intensity of blue and yellow, and the "b" indicator represents the intensity of green and red. Considering that all colors are the result of the ratio of red, yellow and blue, using these three indicators it is possible to determine all

shades of colors down to the smallest differences. The "L" value ranges from 0 to 100, with 0 indicating complete black (absence of light) and 100 complete white. The "a" and "b" values can have a + and - sign, and range from - 60 to + 60.

Fruits were prepared for biochemical analysis by making fruit pulp. After being in a cold chamber, a sample was taken from the pulp for process of determining antioxidant activity. Determination of antioxidant activity was performed by the method of scavenging free stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Sample preparation for determination of antioxidant activity was performed according to the modified method of Tehrani et al. (2011). Radical inhibition by antioxidants present in the sample was monitored spectrophotometrically (UV-VIS, Shimadzu 1240 mini; Shimadzu Corporation, Kyoto, Japan), at a wavelength of 517 nm and was expressed as the effective concentration (EC50), and that is concentration at which 50% of DPPH radicals were scavenged.



Fig. 3 Liquid solutions prepared for the process of determination of antioxidant potential

Results are presented with standard descriptive measures (average and standard deviation, $\bar{x} \pm SD$). Comparison of measured characteristics was conducted with general linear models and where appropriate the post-hoc tests were used. Graphs and biometrical analysis were done with assistance of software package SPSS 22 (IBM, 2013). Statistical significance of differences was set at $p < 0.05$.

RESULTS AND DISCUSSION

The results are presented in accordance to the set research goals of researching the pale-coloured traditional varieties. Characteristics measured and presented with discussion are antioxidant capacity and the Lab of the studied varieties.

Antioxidant Capacity and EC50 Dynamics

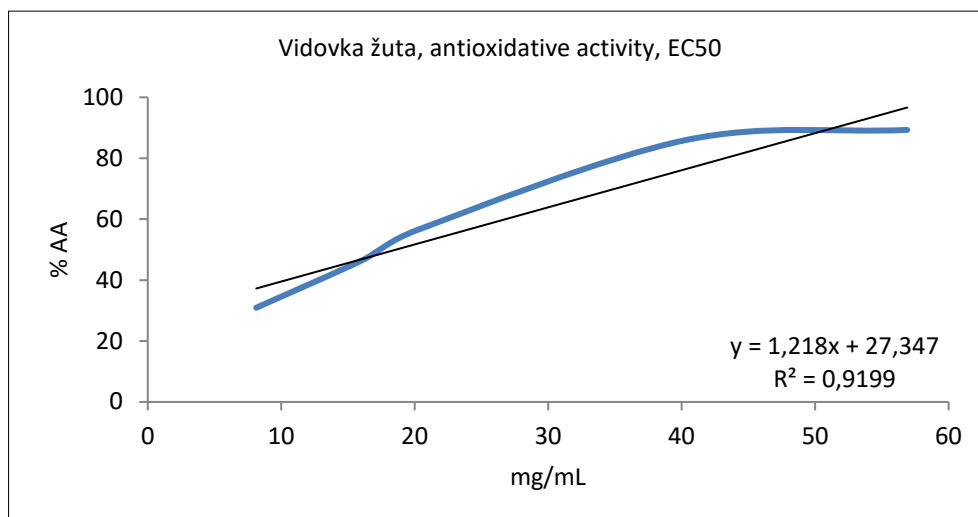
In Table 1 are presented antioxidant capacity and EC50 dynamics for the studied varieties

Table 1. Studied traditional varieties antioxidative capacity with regression and R values

Variety	Regression Equation	R ² Value	Calculated EC50 (mg/mL)
Vidovka žuta	$y = 1.218x + 27.347$	0.9199	18.6
Bjeličnik	$y = 1.1339x + 22.572$	0.9814	24.19
Kanada švabica	$y = 1.2521x + 10.099$	0.9867	31.87

'Vidovka žuta'

The antioxidant potential of the traditional variety 'Vidovka žuta' was quantified by determining the concentration of the extract required to inhibit the DPPH free radicals. The relationship between the concentration and the percentage of antioxidant activity was analyzed using linear regression (Graph 1).

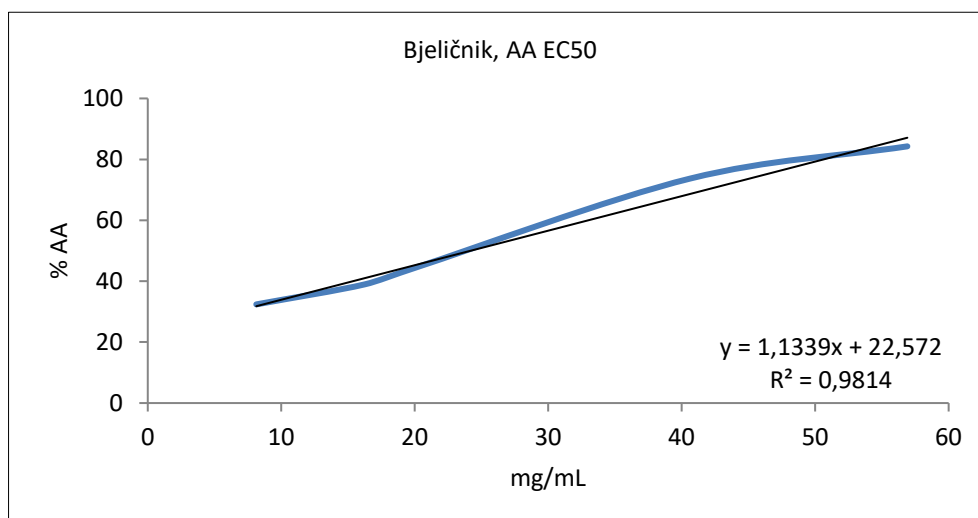


Graph 1. Vidovka žuta, antioxidative activity regression and R values

Results indicate a strong linear correlation within the tested concentration range. However, a closer inspection of the response curve (Figure 1) reveals that the antioxidant activity begins to reach a saturation point (plateau) at approximately 45 mg/mL, where the inhibition reaches roughly 90%. This suggests that 'Vidovka žuta' possesses highly reactive secondary metabolites that are effective even at relatively low concentrations. The EC₅₀ value of 18.60 mg/mL places 'Vidovka žuta' at the top of the studied varieties for antioxidant potency among pale apple varieties. In the context of pomological research in the Republic of Srpska, this variety represents a potential apple genetic resource for functional nutrition.

'Bjeličnik'

Antioxidant potential of the traditional variety 'Bjeličnik' was determined by calculating the EC₅₀ value from the linear regression of its radical scavenging activity. Among the varieties collected from the study 'Bjeličnik' demonstrated a moderate to high efficiency in neutralizing DPPH radicals.

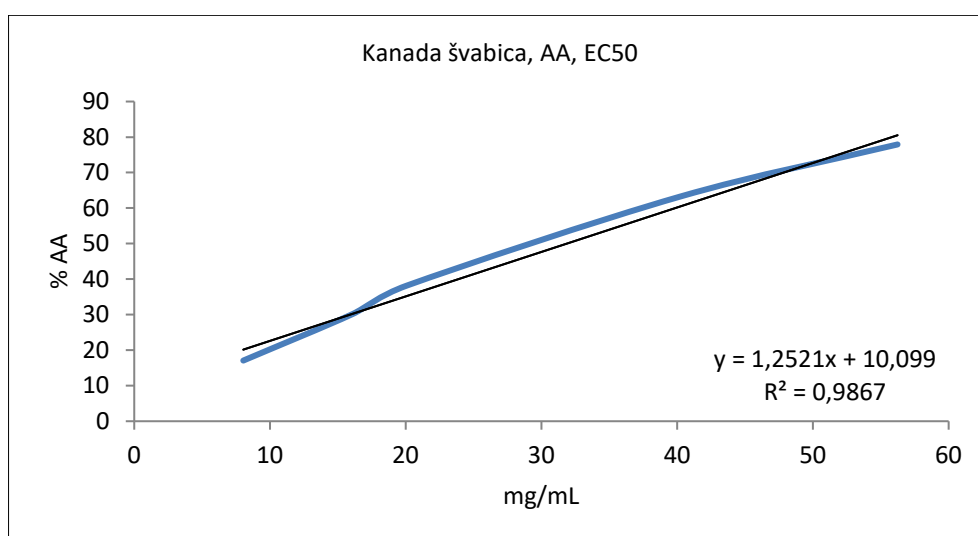


Graph 2. Bjeličnik, antioxidative activity regression and R values

Regression analysis shows an exceptionally high linearity ($R^2=0.9814$), indicating a very stable and predictable dose-response relationship. Unlike 'Vidovka žuta', which showed an early plateau, the 'Bjeličnik' extract maintains a steady increase in inhibition across the tested concentration range. To achieve 50% inhibition, a concentration of 24.19 mg/mL is required. 'Bjeličnik' is an iconic variety in the pomology of Republika Srpska, named for its characteristic pale, almost white skin. This morphological trait is deeply reflected in its chemical and colorimetric data.

'Kanada švabica'

Antioxidant potential of the variety 'Kanada švabica', also sourced from the research collection, was quantified using the same DPPH radical scavenging methodology. Among the three pale varieties studied, 'Kanada švabica' required the highest concentration of extract to achieve significant radical inhibition.



Graph 3. Kanada švabica antioxidative activity regression and R values

Regression analysis for 'Kanada švabica' shows the highest linearity among all tested varieties ($R^2 = 0.9867$), indicating an extremely consistent and predictable relationship between concentration and activity. However, the calculated EC50 of 31.87 mg/mL suggests a lower relative antioxidant potency compared to 'Vidovka žuta' and 'Bjeličnik'.

Fruit Color Analysis (CIE L*a*b*)

Analysis (Table 2) of lightness (L) indicates that all three varieties are statistically distinct from one another ($p < 0.001$). Bjeličnik is significantly lighter than the others (a), confirming its phenotype as the "white" apple of the collection. Kanada švabica is significantly darker than the other two (c).

Analysis of red/green (a*) indicated there is small difference between Vidovka žuta and Bjeličnik, indicating they both share a similar greenish background. Kanada švabica is significantly different, as its color oscillates closer to the neutral/red side. Due to somewhat larger variations formal statistical analysis did not take place for this characteristic.

Yellowness (b*) indicated that Vidovka žuta and Kanada švabica are statistically similar in their yellow intensity (both share group 'a'), while Bjeličnik is significantly less yellow ('c').

Table 2. Lab measures for studied varieties with p-values and grouping from post-hoc test

Lab measures	Variety	Mean	Grouping	SD	p-value (GLM)
L* (Lightness)	Bjeličnik	72.59	a	1.67	<0.001
	Vidovka žuta	68.04	b	3.59	
	Kanada švabica	54.25	c	4.01	
a* (Red/Green)	Kanada švabica	-0.06	a	5.14	-
	Bjeličnik	-4.45	b	1.33	
	Vidovka žuta	-5.13	c	3.4	
b* (Yellow/Blue)	Vidovka žuta	42.06	a	5.45	<0.001
	Kanada švabica	40.65	a	6.7	
	Bjeličnik	23.08	c	2.42	

Color and AA capacity

In studied traditional apple varieties of the Republic of Srpska, "paleness" (L*) does not indicate a lack of bioactive potential. 'Bjeličnik', the lightest variety (L* = 72.59), maintains a very low Coefficient of Variation (CV = 2.30%) for lightness, suggesting high phenotypic stability. While it is visually the palest in color as its EC50 is notably better than that of the darker 'Kanada švabica'. This could indicate that colorless phenolic acids (e.g., chlorogenic acid) are the primary drivers of antioxidant activity in these "white/yellow" apples.

The most potent variety, 'Vidovka žuta', exhibits the strongest relation between its yellow hue (b* = 42.06) and its radical scavenging activity. This suggests that the pigments responsible for the yellow color, likely quercetin glycosides (flavonols), provide a dual benefit: visual attractiveness and related functional protection.

CONCLUSION

Results derived from the studied varieties of Srpska prove that traditional apples are very interesting with values of antioxidant potential, and it is necessary to extend the research when it comes to antioxidant components in samples of fruits of traditional varieties. The high uniformity in 'Bjeličnik' and the superior potency of 'Vidovka žuta' justify their potential use in breeding programs targeting increased functional value in modern apple cultivars.

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REFERENCES

- Asma, U., Morozova, K., Ferrentino, G., & Scampicchio, M. (2023). Apples and Apple By-Products: Antioxidant Properties and Food Applications. *Antioxidants*, 12 (7), 1456. <https://doi.org/10.3390/antiox12071456>
- Arias, A., Feijoo, G., & Moreira, M.T. (2022). Exploring the Potential of Antioxidants from Fruits and Vegetables and Strategies for Their Recovery. *Innovative Food Science and Emerging Technologies*, 77, 102974. <https://doi.org/10.1016/j.ifset.2022.102974>

- Commisso, M., Bianconi, M., Poletti, S., Negri, S., Munari, F., Coledo, S., & Guzzo, F. (2021). Metabolomic Profiling and Antioxidant Activity of Fruits Representing Diverse Apple and Pear Cultivars. *Biology*, 10(5), 380. <https://doi.org/10.3390/biology10050380>
- Gul, M., Akpinar, G., Demican, V., Yilmaz, H., Bal, T., Arici, E., Polat, M., Şan, B., Eraslan, F., & Kart, C. (2014). Economic Analysis of Early-Warning System in Apple Cultivation: A Turkish Case Study. *Custos E Agronegocio Line*, 10, 165–179.
- Hyson, D.A. (2011). A comprehensive review of apples and apple components and their relationship to human health. *Advances in Nutrition*, 2(5), 408–420. <https://doi.org/10.3945/an.111.000513>
- Katz, I.H., Nagar, E.E., Okun, Z., & Shpigelman, A. (2020). The Link between Polyphenol Structure, Antioxidant Capacity and Shelf-Life Stability in the Presence of Fructose and Ascorbic Acid. *Molecules*, 25(1), 225. <https://doi.org/10.3390/molecules25010225> .
- Koschinek, J., Wolfram, T., Stockl, A., & Bohm, V. (2018). Polyphenolic Compounds Analysis of Old and New Apple Cultivars and Contribution of Polyphenolic Profile to the In Vitro Antioxidant Capacity. *Antioxidants*, 7(1), 20. <https://doi.org/10.3390/antiox7010020>
- Mertoglu, K., Akkurt, E., Evrenosoglu, Y., Colak, A.M., & Esatbeyoglu, T. (2022). Horticultural Characteristics of Summer Apple Cultivars from Turkey. *Plants*, 11(6), 771. <https://doi.org/10.3390/plants11060771>
- Mićić N., & Đurić G., (2020). Voćarstvo II.
- Patocka, J., Bhardwaj, K., Klimova, B., Nepovimova, E., Wu, Q., Landi, M., Kuca, K., Valis, M., & Wu, W. (2020). *Malus domestica*: A Review on Nutritional Features, Chemical Composition, Traditional and Medicinal Value. *Plants*, 9(11), 1408. <https://doi.org/10.3390/plants9111408>
- Tehrani, M., Chandran, S., Sharif Hossain, A.B.M., & Nusrulhaq-Boyce, A. (2011). Postharvest physico-chemical and mechanical changes in jambu air (*Syzygium aqueum* Alston) fruits. *Australian Journal of Crop Science*, 5(1), 32–38.
- Wang, Z., Barrow, C.J., Dunshea, R.D., & Suleria, H.A. (2021). A Comparative Investigation on Phenolic Composition, Characterization and Antioxidant Potentials of Five Different Australian Grown Pear Varieties. *Antioxidants*, 10(2), 151. <https://doi.org/10.3390/antiox10020151>
- Wolfe, K., Wu, X., & Liu, R.H. (2003). Antioxidant activity of apple peels. *Journal of Agriculture and Food Chemistry*, 51(3), 609-614. <https://doi.org/10.1021/jf020782a>

Organic vs. conventional viticulture: Implications for stilbene content in grapes

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Abstract

In contemporary plant protection, increasing attention is directed toward plants' natural defence mechanisms, particularly to reduce reliance on synthetic pesticides and promote sustainable production systems. Grapevine exhibits a highly developed secondary metabolism, within which stilbenes, most notably *trans*-resveratrol and its glycoside *trans*-polydatin, are recognised as key components of induced resistance. This study aimed to evaluate the content of these stilbenes in grapes of international grapevine varieties cultivated under organic and conventional production systems, and to examine the influence of cultivar and cultivation type on the plant's immune system. The 2023 growing season was characterised by alternating drought and rainy periods, conditions favourable for pathogen development and activation of plants' defence mechanisms. Healthy grape samples were collected at the harvest period in the same year, according to variety-specific Brix values, and analysed using a previously optimised and validated SPE-HPLC-DAD method. The results showed pronounced differences in stilbene content depending on the production system, with organically grown grapes generally exhibiting higher concentrations of both *trans*-resveratrol and *trans*-polydatin. Under organic production, *trans*-polydatin levels in grapes ranged from 0.24 µg/kg (Sauvignon blanc) to 12.49 µg/kg (Merlot), while *trans*-resveratrol concentrations varied from 0.30 µg/kg (Muscat Italia and Chardonnay) to 1.12 µg/kg (Sauvignon blanc). When cultivar type was considered, clear differences were observed between red and white grapevine varieties, with red ones generally showing higher stilbene concentrations, reflecting a more pronounced secondary metabolic response. An exception was Sauvignon blanc, with higher stilbene levels under conventional production, likely due to cultivar-specific response to chemical stress. These findings highlight the adaptive nature of stilbene biosynthesis and support their relevance within integrated and sustainable plant protection strategies.

Keywords: grapes, stilbenes, resveratrol, organic production, conventional production

INTRODUCTION

Modern viticulture is characterised by different production systems, among which conventional and organic stand out. Conventional viticulture is characterised by the intensive use of synthetic plant protection products (PPPs), mineral fertilisers, mechanisation, and irrigation (Le Campion et al., 2020). Although it enables high and stable yields, the long-term use of these inputs is associated with negative effects, such as the accumulation of pesticide residues in grapes and the environment, the development of pathogen resistance to PPPs, and soil degradation (Poudel et al., 2002; Nash et al., 2010).

In response to these challenges, there is a growing interest in organic viticulture, which involves the use of a limited number of permitted agrochemicals, the implementation of preventive and biological

protection measures, and the selection of grapevine varieties resistant to economically significant pathogens (Alexandrov, 2019; Vukosavljević et al., 2016; Provost & Pedneault, 2016). The increasing demand for organic grapes and wine further drives this trend, although organic production faces limitations, including the conversion period, potentially lower yields, and greater dependence on agro-ecological factors (Cravero, 2019; Lorenz & Lal, 2016).

Given that different cultivation systems impose varying levels of stress due to divergent protection strategies, they are expected to influence the synthesis of these natural defence compounds. Therefore, studying resveratrol and polydatin content in grapes from different production systems is particularly important for understanding grapevine resistance mechanisms and fruit quality.

The differences between these two production systems and their influence on the grapevine are particularly pronounced under climate change, reflected in rising temperatures, extended growing seasons, and earlier grape ripening, affecting yields (Lorenzo et al., 2013; Filimon et al., 2024). Climatic changes also promote the spread of harmful organisms and more intensive development of phytopathogens, especially during alternating dry and wet periods and milder winters (Roques et al., 2010).

In these conditions, the natural defence mechanisms of the grapevine acquire special importance, enabling adaptation to stress and preservation of production stability. This phenomenon is more pronounced in organic systems due to targeted plant protection strategies and increased pathogen exposure, which stimulate natural defence mechanisms. Such responses are often manifested as enhanced stilbene biosynthesis, particularly of resveratrol and its glycoside polydatin.

The highest concentrations of these compounds are found in seeds and grape skin, and their synthesis is intensified under biotic and abiotic stresses, notably infections caused by phytopathogenic fungi, especially *Botrytis cinerea* (Jeandet et al., 1995; Hasan and Bae, 2017). Biosynthesis begins following infection recognition and activation of signalling pathways, and higher synthesis capacity is often associated with more resistant grape varieties (Gutiérrez-Gamboa et al., 2021; Šušnjar et al., 2022).

Given that different cultivation systems impose varying levels of stress due to divergent protection strategies, they are expected to influence the synthesis of these natural defence compounds. Therefore, studying resveratrol and polydatin content in grapes from different production systems is particularly important for understanding grapevine resistance mechanisms and fruit quality.

MATERIAL AND METHODS

Monitoring of resveratrol and polydatin content was carried out in the Republic of Serbia during the 2023 harvest period. The *trans* isomers of these compounds were chosen for analysis due to their greater abundance compared to *cis* isomers, more pronounced biological activity, and commercial availability. Grape sampling was carried out in the localities of Tavankut, Palić, Sremski Karlovci, Čortanovci, Lozovik, and Jovac, where the tested grape varieties were grown in parallel in organic and conventional systems. To minimise the influence of external factors on results, each variety was sampled under as similar agroecological conditions as possible in both production systems. For each variety and production system, one sample with an average weight of 1 kg was taken. The samples were labelled, packed in polypropylene bags, and stored under controlled conditions until analysis.

Right before analysis, 60 g of frozen berries of each sample were homogenised in methanol with 0.1% HCOOH and then extracted using solid phase extraction (SPE). The obtained extracts were analysed using the high-performance liquid chromatography (HPLC) method, and the results were expressed in mg/kg of fresh mass of grapes.

RESULTS AND DISCUSSION

Statistical analysis of the obtained values revealed a statistically significant difference between the examined production systems. In 5 of 6 grape samples, a higher content of *trans*-polydatin was found in organically produced grapes, compared to conventionally produced ones (Table 1). Varieties *Merlot* (12,494 mg/kg) and *Cabernet Franc* (10,628 mg/kg) stand out the most. The variety *Sauvignon blanc*

deviated from this rule, with a 3 times higher content of this compound in conventionally produced grapes, which is additionally reflected in the negative t-value.

Table 1. Content of *trans*-polydatin in conventionally and organically produced grapes

<i>Trans</i> -polidatin	$\bar{X}$ [mg/kg] (OP)	$\bar{X}$ [mg/kg] (CP)	<i>t</i> -value	<i>p</i> -value
Cabernet franc	10.628 ± 0.115	3.689 ± 0.060	106.562	< 0.001
Merlot	12.494 ± 0.157	1.681 ± 0.054	130.402	< 0.001
Muscat Hamburg	2.619 ± 0.055	2.271 ± 0.055	8.897	< 0.001
Chardonnay	0.390 ± 0.016	0.223 ± 0.030	9.667	< 0.001
Sauvignon blanc	0.24 ± 0.002	0.64 ± 0.011	-72.908	< 0.001
Muscat Italia	0.336 ± 0.002	0.289 ± 0.016	5.733	0.001

*OP- organic production; CP- conventional production

Table 2 shows the average values of the content of *trans*-resveratrol in grape varieties from the examined production systems, as well as the results of the t-test for independent samples. Statistically significant differences, as in the content of *trans*-polydatin, were observed in all varieties ($p < 0.001$), whereby almost all varieties also recorded higher values of *trans*-resveratrol within OP. The exception in this case was the variety Sauvignon blanc, with a higher content of *trans*-resveratrol in conventionally produced grapes.

Table 2. Content of *trans*-resveratrol in conventionally and organically produced grapes

<i>Trans</i> -resveratrol	$\bar{X}$ [mg/kg] (OP)	$\bar{X}$ [mg/kg] (CP)	<i>t</i> -value	<i>p</i> -value
Cabernet franc	0.383 ± 0.019	0.131 ± 0.006	24.872	< 0.001
Merlot	0.474 ± 0.025	0.157 ± 0.008	23.918	< 0.001
Muscat Hamburg	0.627 ± 0.011	0.208 ± 0.004	69.036	< 0.001
Chardonnay	0.299 ± 0.012	0.058 ± 0.008	33.350	< 0.001
Sauvignon blanc	1.114 ± 0.012	1.786 ± 0.008	-91.863	< 0.001
Muscat Italia	0.299 ± 0.027	0.073 ± 0.052	7.7	0.001

*OP- organic production; CP- conventional production

Statistical analysis confirmed that for most varieties there are significant differences in the accumulation of these compounds between cultivation systems, which indicates that the type of production has an important, but not the only, influence on their biosynthesis (Baveresco et al., 1997).

For most varieties, higher levels of *trans*-polydatin were found in organically produced grapes, which is consistent with the claim that organic cultivation, due to reduced pesticide use and greater exposure of plants to stress, can induce stilbene synthesis (Levite et al., 2000). Similar tendencies have been reported in studies indicating that organically produced grapes generally accumulate more total polyphenols, including resveratrol and polydatin (Mulero et al., 2010; Hasanaliyeva et al., 2021).

In this research, Merlot and Cabernet franc varieties stood out, with significantly higher *trans*-polydatin content in organically produced grapes. In contrast, opposite tendencies were noted in the Sauvignon blanc variety. Such deviations confirm that genotypic characteristics, together with agro-ecological conditions and chemical-induced stress, can significantly affect stilbene accumulation.

In the case of *trans*-resveratrol, the same pattern was observed with the same scenario, variety Sauvignon blanc, which additionally confirms the fact that genotypic characteristics can have a decisive influence, but also that chemical treatments within the conventional cultivation system can cause stress that will stimulate stilbene biosynthesis (de Andrade et al., 2021). In addition, it was observed that the

content of *trans*-polydatin in the tested varieties is generally higher than the content of *trans*-resveratrol, which is in accordance with the claims that polydatin is the dominant, reserve form of resveratrol in grapes, especially its *trans* isomer, and that as such it represents a reserve form of resveratrol (Vrhovsek et al., 1997; Romero-Perez et al., 1999). However, the observed exception in the Sauvignon blanc variety suggests that genotypic characteristics, agroecological factors, and their interaction with the production system, particularly the response to chemical stress in certain varieties, may significantly modify the biosynthetic potential of plants and contribute to variations in the intensity of stilbene synthesis.

CONCLUSION

The research results indicated statistically significant differences in the content of *trans*-polydatin and *trans*-resveratrol between organic and conventional growing systems. In most tested varieties, a higher stilbene content was found in organically produced grapes, which is desirable from the aspect of plant protection, as it indicates a higher level of natural plant defence. The Merlot and Cabernet franc varieties stood out in particular, as they showed a greater potential for stilbene accumulation and thus a more pronounced defensive capacity. The exception was the variety Sauvignon blanc, where the higher content of stilbene in conventional production indicated the importance of genotypic traits and different reactions to stressful conditions. The obtained results confirm that a higher content of stilbene can represent an important indicator of the natural resistance of the grapevine and an important criterion in the selection of varieties and cultivation systems in order to improve sustainable plant protection. At the same time, these results point to the need for further research into stilbenes as potential natural protective agents in modern plant protection strategies.

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REFERENCES

- Alexandrov, E. (2019). Organic viticulture: real opportunities for implementation. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 19 (1), 25–30.
- Cravero, M. C. (2019). Organic and biodynamic wines quality and characteristics: A review. *Food Chemistry*, 295, 334–340. <https://doi.org/10.1016/j.foodchem.2019.05.149>
- De Andrade, R. B., Machado, B. A. S., Barreto, G. d. A., Nascimento, R. Q., Corrêa, L. C., Leal, I. L., Tavares, P. P. L. G., Ferreira, E. d. S., & Umsza-Guez, M. A. (2021). Syrah Grape Skin Residues Have Potential as Source of Antioxidant and Anti-Microbial Bioactive Compounds. *Biology*, 10 (12), 1262. <https://doi.org/10.3390/biology10121262>
- Filimon, R. M., Bunea, C. I., Filimon, R. V., Bora, F. D., & Damian, D. (2024). Long-Term Evolution of the Climatic Factors and Their Influence on Grape Quality in Northeastern Romania. *Horticulturae*, 10 (7), 705. <https://doi.org/10.3390/horticulturae10070705>
- Gutiérrez-Gamboa, G., Mateluna-Cuadra, R., Díaz-Gálvez, I., Mejía, N., & Verdugo-Vásquez, N. (2021). Methyl jasmonate applications in viticulture: a tool to increase the content of flavonoids and stilbenes in grapes and wines. *Horticulturae*, 7 (6), 133. <https://doi.org/10.3390/horticulturae7060133>
- Hasan, M. M., & Bae, H. (2017). An overview of stress-induced resveratrol synthesis in grapes: perspectives for resveratrol-enriched grape products. *Molecules*, 22 (2), 294. <https://doi.org/10.3390/molecules22020294>
- Hasanaliyeva, G., Chatzidimitrou, E., Wang, J., Baranski, M., Volakakis, N., Pakos, P., Seal, C., Rosa, E. A. S., Markellou, E., Iversen, P. O., Vigar, V., Willson, A., Barkla, B., Leifert, C., & Rempelos, L. (2021). Effect of Organic and Conventional Production Methods on Fruit Yield and Nutritional Quality Parameters in Three Traditional Cretan Grape Varieties: Results from a Farm Survey. *Foods*, 10 (2), 476. <https://doi.org/10.3390/foods10020476>

- Jeandet, P., Bessis, R., Sbaghi, M., & Meunier, P. (1995). Production of the phytoalexin resveratrol by grape as a response to *Botrytis* attack under natural conditions. *Journal of Phytopathology*, 143, 135–139. <https://doi.org/10.1111/j.1439-0434.1995.tb00246.x>
- Le Campion, A., Oury, F. X., Heumez, E., & Rolland, B. (2020). Conventional versus organic farming systems: dissecting comparisons to improve cereal organic breeding strategies. *Organic Agriculture*, 10, 63–74. <https://doi.org/10.1007/s13165-019-00249-3>
- Levite, D., Adrian, M., & Tamm, L. (2000). Preliminary Results on Contents of Resveratrol in Wine of Organic and Conventional Vineyards. Proceedings of the 6th International Congress on Organic Viticulture, Basel (Suisse), 256–257.
- Lorenz, K., & Lal, R. (2016). Advances in Agronomy, Chapter Three - Environmental Impact of Organic Agriculture, Editor: Sparks, D. L. Academic Press, 139, 99–152. <https://doi.org/10.1016/bs.agron.2016.05.003>
- Lorenzo, M. N., Taboada, J. J., Lorenzo, J. F., & Ramos, A. M. (2013). Influence of climate on grape production and wine quality in the Rías Baixas, north-western Spain. *Regional Environmental Change*, 13 (4), 887–896. <https://doi.org/10.1007/s10113-012-0387-1>
- Mulero, J., Pardo, F., & Zafrilla, P. (2010). Antioxidant activity and phenolic composition of organic and conventional grapes and wines. *Journal of Food Composition and Analysis*, 23 (6), 569–574. <https://doi.org/10.1016/j.jfca.2010.05.001>
- Nash, M. A., Hoffmann, A. A., & Thomson, L. J. (2010). Identifying signatures of chemical applications on indigenous and invasive nontarget arthropod communities in vineyards. *Ecological Applications*, 20, 1693–1703. <https://doi.org/10.1890/09-1065.1>
- Poudel, D. D., Horwath, W. R., Lanini, W. T., Temple, S. R., & van Bruggen, A. H. C. (2002). Comparison of soil N availability and leaching potential, crop yields, and weeds in organic, low-input, and conventional farming systems in northern California. *Agriculture, Ecosystems & Environment*, 90 (2), 125–137. [https://doi.org/10.1016/S0167-8809\(01\)00196-7](https://doi.org/10.1016/S0167-8809(01)00196-7)
- Provost, C., & Pedneault, K. (2016). The organic vineyard as a balanced ecosystem: Improved organic grape management and impacts on wine quality. *Scientia Horticulturae*, 208, 43–56. <https://doi.org/10.1016/j.scienta.2016.04.024>
- Romero-Pérez, A. I., Ibern-Gómez, M., Lamuela-Raventós, R. M., & de La Torre-Boronat, M. C. (1999). Piceid, the major resveratrol derivative in grape juices. *Journal of Agricultural and Food Chemistry*, 47 (4), 1533–1536. <https://doi.org/10.1021/jf981024g>
- Roques, A., Kenis, M., Lees, D., Lopez-Vaamonde, C., Rabitsch, W., Rasplus, J. Y., & Roy, D. B., (2010). Alien terrestrial arthropods of Europe, Chapter 5. BIORISK - Biodiversity and Ecosystem Risk Assessment, 4 (1), 1028.
- Šušnjar, A., Šunjka, D., Bošković, D., Lazić, S., & Vuković, S. (2022). Resveratrol in grapes—benefits and potential use. *Agrofor*, 7 (2), 110–122. <https://doi.org/10.7251/AGRENG2202110S>
- Vrhovsek, U., Wendelin, S., & Eder, R. (1997). Effects of Various Vinification Techniques on the Concentration of cis- and trans-Resveratrol and Resveratrol Glucoside Isomers in Wine. *American Journal of Enology and Viticulture*, 48 (2), 214–219. <https://doi.org/10.5344/ajev.1997.48.2.214>
- Vukosavljević, V., Žunić, D., Todić, S., & Matijašević, S. (2016). Organic viticulture in world, Serbia, and the region. *Acta Agriculturae Serbica*, 21 (42), 155–166.

Assessment of the nutritional status of Simmental cows based on milk chemical composition

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Abstract

The aim of this study was to determine, based on the analysis of milk constituents, whether cows were fed in accordance with their nutritional requirements. Milk urea concentration is a reliable indicator of the protein supply to the body, as well as the balance between energy and protein in the cows diet. Urea concentration can therefore be used to assess whether cows are appropriately fed according to their needs. Milk urea concentration is influenced by multiple factors, including breed, diet, stage of lactation, body weight, daily milk yield, season, age of the animal, parity, milking interval, environmental conditions, and health status. In the Republic of Srpska, milk sampling is performed monthly on all dairy farms to analyze protein and fat content, urea concentration, lactose content, dry matter content, and somatic cell count. All cows on the studied farm were kept under standard farming conditions and fed the same diet. The average milk composition was as follows: milk fat 4.29%, protein 3.74%, fat/protein ratio 1.15, urea concentration 18.24 mg/dl, and milk urea nitrogen (MUN) 8.39 mg/dl. Considering the average results over the 12 month period, it can be observed that the cows were generally fed in accordance with their nutritional requirements, but when considering the analysis for each month individually, it is clear that in some months the cows were not fed in accordance with their nutritional requirements. The study results showed that the average values of milk urea concentration and other milk constituents indicate a properly balanced diet for dairy cows, with observed monthly variations confirming the importance of continuous monitoring of milk composition.

Keywords: milk urea, milk urea nitrogen, dairy cattle, Simmental cow

INTRODUCTION

During the feeding of dairy cattle, one of the main goals is to maximize the utilization of the genetic potential of a specific animal. Increased genetic potential for milk production in dairy cows is associated with reduced fertility (Roy et al., 2011). Milk is produced in the secretory cells of the mammary glands in female mammals during the lactation period. The composition of milk includes: water, proteins, fat, lactose, mineral matters, and vitamins (Walstra et al., 2006). Depending on the breed, age of the animal, number of calvings, lactation stage, nutrition, season, milking interval, physical environment (ambient conditions), as well as the health status of the animal, these ingredients can vary significantly (Haile et al., 2008; Konjačić et al., 2010; Guetouache et al., 2014; Khastayeva et al., 2021; Goyal et al., 2024). In order to fully utilize the genetic potential and achieve maximum production of good quality milk, it is necessary to feed the cows a balanced diet, that is, it is necessary to determine their actual needs. When increasing the content of crude proteins in the diet, the amount of protein degraded in the rumen also increases, and if there is more degradable protein in the rumen than the needs of the microorganisms, large amounts of ammonia are created, which is absorbed into the blood, converted into urea in the liver, and excreted through urine (Olmos Colmenero and Broderick, 2006). For this reason, a good assessment of the cows' protein needs is very important for optimizing production performance and reducing nitrogen excretion through urine (Lacková et al., 2024). By placing an excessive amount of crude proteins in the diet, we also significantly increase the

cost of production because protein feeds are expensive. With a properly balanced diet in protein and energy nutrition, the health and productivity of animals can be improved (Godden et al., 2001; Roy et al., 2011). Rumen microorganisms need both NH_3 and energy (from fermentable carbohydrates) at the same time to grow efficiently, so protein alone is not useful without energy and energy alone is not inefficient without protein. The greatest efficiency of protein utilization is achieved when the amount of nitrogen in the feed is aligned with the needs of microbes in the rumen and ruminant tissues, and this is reflected in the concentration of urea in the blood and milk (Baker et al., 1995). By regular analysis of milk for urea concentration, we can avoid many problems both in cow reproduction and from the aspect of the cost price of cow feeding. Broderick and Clayton (1997) state numerous benefits from monitoring urea in milk: excessive protein intake can impair the reproductive health of cows, consumption of excess crude proteins increases energy needs, protein supplements are expensive diet ingredients and should be used rationally, excessive nitrogen excretion has a negative impact on the natural environment.

Milk protein contains true protein and non-protein nitrogen compounds (NPN). Milk contains about 95% true protein, while the remaining 5% consists of NPN (Roy et al., 2011). The end product of protein metabolism is urea, and based on the urea content in milk, we can determine the energy status of dairy cows, as well as the supply of nitrogenous substances in the feed (Horky, 2014).

An increased urea content in milk is an indicator of excess protein in the diet of dairy cows (Hojman et al., 2004) and a decrease in the energy value of the ration. According to Kubesova et al. (2009), the physiological value of urea concentration in milk ranges from 20 to 30 mg/100 ml of milk, while Babnik et al. (2004) state that urea content in the range of 15 to 30 mg/100 ml is considered physiological values. According to Siatka et al. (2020), the optimal urea concentration for high-yielding dairy cows (Holstein Friesian) ranges between 150 and 300 mg/l, and Horky (2014) states that in cows with higher production, up to 35 mg/100 ml of milk is tolerated. Šamanc et al. (2006) state that if the urea concentration in milk is less than 4.0 mmol/l and the protein concentration is higher than 32.0 g/l, the cow is considered to be fed in accordance with her production needs, i.e., with an adequate supply of energy and protein through the ration. Due to a pronounced lack of energy and reduced activity of rumen microorganisms, the urea concentration increases to between 5 and 10 mmol/l while the protein content drops below 30 g/l (Savić et al., 2011), and in the case of a decrease in the concentration of urea and protein in milk below physiological values, the cause is a lack of protein in the dairy cows diet (Savić et al., 2019). Research shows that cows with a lower urea concentration in milk below 10.0 mg/dl become pregnant more easily, while high urea concentrations above 15.4 mg/dl negatively affect fertility and reduce the chances of successful conception (Rajala-Schultz et al., 2001). Increasing the urea concentration from 12.5 mg/dl to 13.5 mg/dl reduced conception success by up to 5% (Kananub et al., 2020). This is supported by data where Butler et al. (1996) state that a urea concentration in milk higher than 19 mg/dl leads to even 20% lower conception rates in Holstein cows, and according to Chaveiro et al. (2011), at a urea concentration higher than 16 mg/dl, the conception rate was 14% lower than in cows with a lower urea concentration.

Milk protein content depends on whether the microorganisms in the rumen have enough energy available for the synthesis of microbial protein (Shulga et al., 2014). Goyal et al. (2024) state that cow's milk contains from 3.2 to 3.8% protein and from 3.7 to 4.4% milk fat. According to the regulations on milk quality in the Republic of Srpska from 2015, cow's milk must contain at least 3.2% milk fat, at least 3.0% protein, and at least 8.5% non-fat dry matter. According to Roy et al. (2011), in dairy cows, the check of protein supply can be performed in two ways: by monitoring the intake of crude proteins and by checking the urea concentration in milk or blood.

The assessment of diet quality, specifically the supply of animals with easily digestible proteins, energy, and dietary fibers, is possible by calculating the ratio between milk fat and protein, as well as the protein content in milk (Eicher, 2004). If this ratio is greater than 1.5, then lipomobilization and a decrease in protein concentration in milk occur, which is most often a consequence of reduced feed intake, and usually happens at the beginning of lactation due to reduced milk production. In contrast, a reduced ratio of milk fat and protein below 1 is most common in the initial phase of lactation when there is insufficient crude fiber content in the diet and an increased amount of concentrate (Savić et al., 2011).

An increased milk fat content above the physiological limit often indicates the existence of a negative energy balance and lipomobilization, especially in early lactation. Critical values are milk fat content above

45 g/l and protein content below 32 g/l, as well as FPR values (fat to protein ratio) above 1.5 and below 1. An increased FPR value can most commonly be observed at the beginning of lactation, when lipomobilization and a decrease in milk protein concentration occur due to reduced feed intake, or in late lactation, when the milk is "thickened" due to a decrease in the amount of milk produced. A decrease in the FPR value can be observed when the crude fiber content in the diet is insufficient, most often in the initial phase of lactation, when farmers try to compensate for the energy deficit caused by increased energy needs and reduced feed intake by giving larger amounts of concentrate to the cows (Savić et al., 2011; Kirovski et al., 2012). Analysis of milk for urea concentration, protein share, and milk fat is performed once a month on all dairy cow farms in the Republic of Srpska. Based on the obtained results, it is possible to assess whether the cows are properly fed, that is, whether their diet is adequately balanced in terms of energy and protein.

The aim of this paper was, based on the analysis of organic milk ingredients, to determine whether the cows were fed in accordance with their needs.

MATERIAL AND METHODS

This study was conducted on Simmental cows in different lactation stages on an average farm in the Republic of Srpska. Milk sampling for further analysis was performed once a month, specifically 12 times, in the period from January 2024 to December 2024. The number of cows per sampling varied from 10 to 16 depending on the month of analysis. The cows were at different stages of lactation. The average body weight of the cows is about 650 kilograms. The cows were fed a complete total mixed ration (TMR) throughout all stages of lactation. The winter diet consisted of silage, hay, straw, and concentrate, whereas the summer diet included haylage, silage, and concentrate. All cows were in a free stall housing system. The cows were milked twice a day. For analysis, milk samples were taken in the morning, from each cow immediately after milking. The determination of milk fat, protein, lactose, and dry matter content was performed in the laboratory for raw milk control, using a Bentley 150 infrared milk analyzer, while the urea concentration was determined on a Bentley Chemspec 150 Urea Analyzer for Milk. MUN (milk urea nitrogen) was calculated based on the daily milk urea content using the following formula: $MUN (mg/dL) = UREA * 0.46$ (Spiekers & Obermaier, 2012).

Statistical analysis was performed using IBM SPSS Statistics 22. Comparisons of results according to months were done using one-way analysis of variance (ANOVA) followed by the Scheffé post hoc test. In order to see the correctness of the applied nutrition by months, an assessment of the supply of cows with digestible proteins, energy, and structural fibers was presented based on the F/P ratio and protein content in milk, as well as based on the protein and urea content in milk across the analyzed months.

RESULTS AND DISCUSSION

Depending on various factors, the composition of milk can vary more or less. The chemical composition of milk depends on the breed, lactation number, production system, and mostly on the nutrition of the cows. For the purposes of this paper, daily milk yield, milk fat, proteins, and urea were determined in examined cows.

Table 1. Basic statistical parameters of daily milk yield, milk fat, proteins and urea in examined cows.

Parameter	Mean	SD	SE	CV (%)	Minimum	Maximum
Daily milk yield (kg)	19.68	1.50	0.43	7.63	18.01	23.42
Milk fat (%)	4.29	0.67	0.05	15.59	2.14	5.83
Proteins (%)	3.74	0.38	0.03	10.18	2.48	4.94
Fat to protein ratio (FPR)	1.15	0.18	0.01	14.48	0.64	1.55
Urea (mg/dl)	18.24	6.85	0.55	37.54	5.60	38.70
MUN (mg/dl)	8.39	3.15	0.25	37.54	2.58	17.80

Urea – daily urea content (mg/dl); MUN – milk urea nitrogen (mg/dl); SD – standard deviation; SE – standard error; CV – coefficient of variation

Based on the results in Table 1, it can be seen that the values for all 12 months in total for the parameters: milk fat content (4.29%), protein content (3.74%), as well as urea concentration (18.24 mg/dl) are within the limits of physiological values. Optimal daily values of urea concentration in milk range from 15 to 30 mg/dl (Ruska et al., 2017). However, based on the minimum and maximum values, it can be observed that there are deviations from the physiological values. Regarding milk fat, those values range below the physiological limits (2.14%) as well as above the physiological limits (5.83). In the research by Kitonjić (2014) in Simmental cows, according to lactations, the following values for milk fat content were recorded: 37.76 g/l (3.78%) in the first lactation, 41.30 g/l (4.13%) in the second lactation, and 42.15 g/l (4.22%) in the third lactation. Bendelja et al. (2011) recorded a lower value compared to the present study (4.29%) for the average milk fat content in the Simmental breed (4.19%). Uzun and Koc (2025) in their research state an average milk fat content of 3.83%. Kaygisiz and Sahin (2023) state an average milk fat content of 3.60% in the Simmental breed. Falta et al. (2023) reported that the average milk fat content in the Simmental breed was 3.99%.

Also, the protein content for the analyzed period ranges below the physiological values for individual animals in certain months (2.48%) as well as above the physiological values (4.94%). In the research by Kitonjić (2014), lower values for the average protein content were recorded: 35.91 g/l in the first lactation, 35.37 g/l in the second lactation, and 36.18 g/l in the third lactation. Bendelja et al. (2011) recorded a lower average protein content of 3.36% in the Simmental breed compared to present study of 3.74%. Uzun and Koc (2025) state an average protein content of 3.42%. Falta et al. (2023) in their research state an average protein content of 3.60%. Kaygisiz and Sahin (2023) state an average protein content of 3.40% in the Simmental breed. The same authors recorded a milk fat content of 2.95% and a protein content of 3.59 in cows that had acidosis, and a milk fat content of 5.03% and a protein content of 3.17% in cows that had ketosis. The fat/protein ratio is also variable and ranges from 0.64 to 1.55. The urea concentration also ranges below the physiological values for individual animals in certain months at 2.58 (mg/dl) as well as above the physiological values at 38.70 (mg/dl).

According to Gavran et al. (2021), in Simmental cows, the daily milk yield was lower than in present study (19.68 kg) and amounted to 16.13 kg, while Falta et al. (2023) recorded a higher daily production of 27.56 kg, as did Bendelja et al. (2011) at 25.99 kg, while Bittante (2021) recorded 22.0 kg. In the research by Gavran et al. (2021), the urea concentration was higher than in present study (18.24 mg/dl) and amounted to 19.55 mg/dl, and in the research by Bendelja et al. (2011), it was 24.56 mg/100 ml. According to literature reports, different values of urea concentration are taken as physiological values; thus, Marenjak et al. (2004) state that the physiological values of urea concentration in cow's milk range from 10 to 30 mg/dL, and according to Young (2001), those values are from 12 to 16 mg/dl. In the research by Falta et al. (2023), the urea concentration was 25.75 mg/100 ml.

Based on the obtained results shown in Table 2 analyzed by months, it can be seen that there are statistically significant differences ($p < 0.05$) between the analyzed months. The milk fat content in the months of January (4.90%), July (4.40%), September (4.78%), as well as November (4.99%) was above the physiological limits. High milk fat values at the beginning of lactation are most often a consequence of excessive depletion of body reserves characteristic of high-yielding animals, and are an indicator of digestive disorders. If the milk fat content is high at the end of lactation, the most common cause is a decrease in milk production (Gantner and Barać, 2014). According to Savić et al. (2010), in cows where there is an intensive process of lipomobilization, the level of free fatty acids in the blood is elevated, which leads to an increase in their uptake by the mammary gland and an increase in the milk fat content. In cows, an increased milk fat content above 45 g/l and a decreased protein content below 32.0 g/l indicate an energy deficit (Savić et al., 2010). In the research by Khastayeva et al. (2021), Simmental cows in the spring period had a milk fat content of 3.94%, in summer 3.99%, in autumn 4.04%, and in the winter period 4.09%. Chernyavska (2022) states that in Ukraine, in Simmental first calf heifers, the milk fat content was 4.05%, and in adult cows 4.35%. The fat content in Simmental cows in the first lactation was 3.83%, in the second 3.84%, and in the third 3.86% (Budimir et al., 2011). Falta et al. (2023) state for the Simmental breed a milk fat content from December to February of 4.09%, from March to May 4.07%, from June to August 4.15%, and from September to November 4.30%.

Table 2. Mean values for monthly milk production, daily milk yield, milk fat content, protein content, fat to protein ratio, and concentration of daily milk urea, milk urea nitrogen by months of monitoring.

Recording month	Number of cows	Monthly milk yield (kg)	Daily milk yield (kg)	Milk fat (%)	Proteins (%)	Fat to protein ratio (FPR)	Urea (mg/dL)	MUN (mg/dL)
January	16	10056	20.27	4.90 ^a	3.86 ^a	1.27 ^{ae}	30.91 ^a	14.22 ^a
February	16	9947	21.44	4.31 ^{ab}	3.73 ^a	1.16 ^{abe}	18.15 ^{bc}	8.35 ^{bc}
March	16	9480	19.11	4.19 ^{ab}	3.73 ^a	1.12 ^{abe}	16.18 ^{bc}	7.44 ^{bc}
April	15	9075	20.17	3.99 ^b	3.74 ^a	1.07 ^{bc}	14.44 ^{bc}	6.64 ^{bc}
May	15	8893	19.12	3.91 ^b	3.88 ^a	1.01 ^{bd}	12.42 ^c	5.71 ^c
June	15	8788	19.53	3.59 ^b	3.66 ^a	0.98 ^{bd}	19.51 ^{bc}	8.97 ^{bc}
July	10	5711	18.42	4.40 ^{ab}	3.45 ^a	1.28 ^{ace}	22.51 ^{ab}	10.35 ^{ab}
August	11	6140	18.01	4.41 ^{ab}	3.70 ^a	1.20 ^{acde}	19.64 ^{bc}	9.03 ^{bc}
September	12	6647	18.46	4.78 ^a	3.61 ^a	1.33 ^e	18.49 ^{bc}	8.51 ^{bc}
October	11	6531	19.15	4.20 ^{ab}	3.74 ^a	1.14 ^{acde}	16.28 ^{bc}	7.49 ^{bc}
November	11	6302	19.10	4.99 ^a	3.84 ^a	1.30 ^e	15.56 ^{bc}	7.16 ^{bc}
December	12	8711	23.42	4.04 ^{ab}	3.79 ^a	1.06 ^{acd}	13.74 ^{bc}	6.32 ^{bc}

Urea – daily urea content (mg/dL); MUN – milk urea nitrogen (mg/dL);
^{abc} statistically significant differences at $p < 0.05$

When it comes to the protein content, for the months of January (3.86%), May (3.88%), and November (3.84%), the values were also above the physiological limits. No statistically significant differences were observed for the protein content between the analyzed months. In the research by Khastayeva et al. (2021), those values are lower; the protein content in the spring period was 3.32%, in summer 3.45%, in autumn 3.39%, and in the winter period 3.41%. Also, in her research, Chernyavska (2022) states lower values compared to the present study; in Simmental first-calf heifers, the protein content was 3.01%, and in adult cows 3.33%. In the research by Falta et al. (2023), somewhat lower values were also recorded compared to the present study: from December to February 3.69%, from March to May 3.61%, from June to August 3.68%, and from September to November 3.75%.

Based on the results in Table 2, we can see that the fat/protein ratio in most of the analyzed months was optimal, while for the months of April (1.07), May (1.01), June (0.98), and December (1.06), it was below optimal. Statistically highly significant differences ($p < 0.001$) were found between certain analyzed months. Gubanov and Chasovshchikova (2021) in their research state that the average fat/protein ratio for the Simmental breed was 1.18 in January, 1.11 in February, and 1.16 in March, but the minimum and maximum values were outside the optimal values: in January from 0.56 to 2.02, in February from 0.52 to 2.74, and in March from 0.50 to 2.47. If the fat/protein ratio is greater than 1.5:1.0, such a condition indicates a critically high level of consumption of the cow's body fat reserves.

The urea concentration in milk in the month of January was far higher than in other months and amounted to 30.91 mg/dl, followed by July at 22.51 mg/dl, as well as MUN (milk urea nitrogen), which in January amounted to 14.22 mg/dl and in July 10.35 mg/dl. However, no statistically significant differences were found between the urea content in milk in July and January. The reason for this is an abundant supply of degradable proteins as well as an abundant supply of digestible proteins, i.e., energy. This occurs mainly in cows in the late stage of lactation, and it is necessary to reduce the protein content in the diet. Gavran et al. (2021) recorded higher values of urea concentration in milk in the summer period (in July), specifically 26.35 mg/dl, as well as a MUN of 11.62 mg/dl. Contrary to the above, in the research by Marenjak and Poljičak-Milas (2007), a significantly lower urea concentration in milk in the Simmental breed was recorded during the summer months, and the highest in autumn. In the research by Oudah (2009), the lowest urea concentration was in February (29.1 mg/dl) and March (25.2 mg/dl), and the highest in May (36.1 mg/dl) as well as September (34.2 mg/dl). Higher values of urea concentration than those obtained

in present study were reported by Falta et al. (2023): from December to February it amounted to 28.18 mg/100ml, from March to May 25.00 mg/100ml, from June to August 20.62 mg/100ml, and from September to November 24.92 mg/100ml.

Marenjak and Poljičak-Milas (2007) reported a significantly lower urea concentration in the milk of Simmental cows during the summer months, while the highest urea concentration was recorded in autumn. Uzun and Koc (2025) state in their research that the MUN concentration amounted to 15.92 mg/dl. Važić et al. (2020), in their examinations of the metabolic profiles of cows, state statistically significant differences in the blood urea content of cows depending on the feeding season. If the urea concentration in milk is above physiological values in cows, the reason is likely the intake of too much protein; conversely, if the urea concentration in milk is below physiological values, it is an indicator of insufficient protein intake (Roy et al., 2011). According to Johnson and Young (2003), a lower urea concentration in milk can be associated with better utilization of crude proteins in the diet, resulting in more efficient utilization of nitrogen from the feed in the animal's body.

Table 3. Assessment of cows supply with digestible protein, energy, and structural fibers based on the fat-to-protein ratio and milk protein content shown by months.

Fat/protein ratio in milk	Supply of metabolizable protein and energy				Supply of structural fibers
	Low	Optimal	Excessive		
	>1.5	A1M/P	A2M/P	A3M/P	Excessive
	1.1-1.5	B1M/P	B2M/P February, March, July, August, September, October	B3M/P January, November	Optimal
	< 1.1	C1M/P	C2M/P April, June, December	C3M/P May	Low
	< 3.2	3.2-3.8	> 3.8		
Milk protein content (%)					

Table 4. Assessment of cow supply with degradable and digestible proteins based on milk protein and urea content by analyzed months.

Protein content (%)	Supply of degradable proteins				Supply of metabolizable protein and energy
	Low	Optimal	Excessive		
	>3.8	A1P/U May	A2P/U	A3P/U January	Excessive
	3.2-3.8	B1P/U April, December	B2P/U February, March, June, July, August, September, October, November	B3P/U	Optimal
	< 3.2	C1P/U	C2P/U	C3P/U	Low
	< 15	15-30	> 30		
Milk urea concentration (mg/dl)					

Based on the values from Table 3, we can see that most months are in the B2 M/P group, which indicates optimal nutrition, optimal supply of cows with energy, metabolizable protein, and structural fibers. The C2 M/P group means that the cows are optimally provided with metabolizable protein and energy, mostly being animals in whose diet too little structural fiber is represented. Possible causes are too large a proportion of concentrated feed in the diet, then a too rapid transition to diets with a higher proportion of concentrated feed, as well as a too rapid transition from winter to summer diet. Groups B3 M/P and C3 M/P are characterized by an excessive supply of metabolizable protein and energy. Group B3 M/P is characteristic of cows at the end of lactation. The reason is a too abundant diet considering the needs of the cow in that period, meaning that cows receive more energy and protein than they need. Group C3 M/P is also characteristic of cows after the 8th month of lactation. The cause is a diet with a too large proportion of concentrated feed or corn silage and a too small proportion of structural fiber (Gantner and Barać, 2014).

Group B1 P/U indicates a deficiency of rumen-degradable proteins in the diet, and it is necessary to increase them in the ration. This is characteristic of cows in mid and late lactation. Group B2 P/U tells us that the cows are optimally fed with degradable proteins in the diet. Group A3 P/U is characterized by an excess of degradable proteins in the diet. It most commonly occurs in cows in late lactation, and it is necessary to reduce the proportion of protein in the ration.

CONCLUSION

In accordance with the set objective of the study, the adequacy of dairy cow nutrition on the examined farm was determined based on the analysis of organic milk constituents. Based on the obtained results, it can be concluded that the analysis of milk urea concentration, together with milk fat content, protein, and the fat/protein ratio, proved to be a reliable indicator of the body's protein supply and the balance of the energy-to-protein ratio in the diet. The average values obtained over the twelve-month period (milk fat 4.29%, protein 3.74%, fat/protein ratio 1.15, urea concentration 18.24 mg/dl, and MUN 8.39 mg/dl) indicate that the cows' nutrition as a whole was properly balanced and adapted to their production needs. The obtained average values are within physiologically acceptable limits, confirming that the nutrition was properly organized and aligned with the animals' requirements, with adequate microbial protein synthesis in the rumen. However, the analysis of results by individual months showed variations in urea concentration and other parameters, indicating occasional imbalances between the diet and the actual needs of the cows in certain stages of lactation or under the influence of seasonal and production factors. These oscillations point to the need for continuous monitoring of milk composition for timely adjustment of the diet to the production and physiological needs of dairy cows. The results of this study can serve as a basis for improving the nutrition system and optimizing rations in order to achieve higher production efficiency while maintaining the health status of the animals on the examined farm.

REFERENCES

- Babnik, D., Verbič, J., Podgoršek, P., Jeretina, J., Perpar, T., Logar, B., Sadar, M., & Ivanovič B. (2004). Priručnik za vođenje prehrane krav molznica ob pomoći rezultata mlečne kontrole. *Kmetijski inštitut Slovenije, Ljubljana*.
- Baker, L. D., Ferguson, J. D., & Chalupa, W. (1995). Responses in urea and true protein of milk to different protein feeding schemes for dairy cows. *Journal of Dairy Science*, 78(11), 2424-2434. [https://doi.org/10.3168/jds.S0022-0302\(95\)76871-0](https://doi.org/10.3168/jds.S0022-0302(95)76871-0)
- Bendelja, D., Prpić, Z., Mikulec, N., Ivkić, Z., Havranek, J., & Antunac, N. (2011). Milk urea concentration in Holstein and Simmental cows. *Mljekarstvo*, 61 (1), 45-55.
- Bittante, G. (2022). Effects of breed, farm intensiveness, and cow productivity on infrared predicted milk urea *Journal of Dairy Science*, 105(6), 5084-5096. <https://doi.org/10.3168/jds.2021-21105>
- Broderick, G. A., & Clayton, M. K. (1997). A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *Journal of Dairy Science*, 80(11), 2964-2970. [https://doi.org/10.3168/jds.S0022-0302\(97\)76262-3](https://doi.org/10.3168/jds.S0022-0302(97)76262-3)
- Budimir, D., Plavšić, M., & Popović-Vranješ, A. (2011). Production and reproduction characteristics of simmental and holstein friesian cows in Semberija area. *Biotechnology in Animal Husbandry*, 27(3), 893-899. <https://doi.org/10.2298/BAH1103893B>
- Butler, W. R., Calaman, J.J., & Beam, S. W. (1996). Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *Journal of Animal Science*, 74(4), 858-865. <https://doi.org/10.2527/1996.744858x>
- Chaveiro, A., Andrade M., Borba A. E.S., & Moreira da Silva, F. (2011). Association between Plasma and Milk Urea on the Insemination Day and Pregnancy Rate in Early Lactation Dairy Cows. *Journal of Physiology and Pharmacology advances*, 1(1), 9-14.
- Chernyavska, T. O. (2022). Research of the qualitative composition of milk of cows of simmental breed. *Animal Breeding and Genetics*, 63, 142-147. <https://doi.org/10.31073/abg.63.12>

- Eicher, R. (2004). Evaluation of the metabolic and nutritional situation in dairy herds: Diagnostic use of milk components. *23rd World Buiatrics Congress, Quebec City, Canada*, 11–16 July. pp 1-6.
- Falta, D., Zapletalova, L., Hanus, O., Kucera, J., Vecera, M., Chaldek Filipcik, R., Kopec, T., & Lategan, F. S. (2023). The interaction between the milk production. milk components with a low frequency of analysis and factors affecting the milk composition in dual-purpose Simmental cows. *Czech Journal of Animal Science*, 68(3), 99-110. <https://doi.org/10.17221/197/2022-CJAS>
- Gantner, V., & Barać Z. (2014). Uzgojno-seleksijski rad u stočarstvu. *Poljoprivredni fakultet Sveučilišta Josipa Jurja Strossmayera u Osijeku*. ISBN:978-953-7871-35-2.
- Gavran, M., Bešlo, D., Gregić, M., Šinka, D., Zvonimir Steiner, Z., & Gantner, V. (2021). Variability of milk urea, milk urea nitrogen, and ammonia emission from dairy Simmental and Holstein cows based on the milk recording month. *Agro-knowledge Journal*, 22(2), 27-35. <https://doi.org/10.7251/AGREN2202027G>
- Guétouache, M., Bettache, G., & Medjekal, S. (2014). Composition and nutritional value of raw milk. *Issues in Biological Sciences and Pharmaceutical Research*, 2(10), 115-122. <https://doi.org/10.15739/ibspr.005>
- Godden, S. M., Lissemore, K. D., Kelton, D. F., Leslie, K. E., Walton, J. S., & Lumsden, J. H. (2001). Relationships Between Milk Urea Concentrations and Nutritional Management, Production, and Economic Variables in Ontario Dairy Herds. *Journal of Dairy Science*, 84(5), 1128-1139. [https://doi.org/10.3168/jds.S0022-0302\(01\)74573-0](https://doi.org/10.3168/jds.S0022-0302(01)74573-0)
- Goyal, M. R., Suvartan, R., & Junaid, A. M. (2024). The chemistry of milk and milk products. Physicochemical Properties, Therapeutic Characteristics, and Processing Methods. Innovations in Agricultural and Biological Engineering. Apple Academic Press exclusively co-publishes with CRC Press, an imprint of Taylor & Francis Group, LLC. ISBN: 978-1-00334-070-6
- Gubanov, M., & Chasovshchikova, M. (2021). Mass fraction of fat, protein, and their ratio in Simmental breed cows' milk. *BIO Web of Conferences* 36, 06013, 10. <https://doi.org/10.1051/bioconf/20213606013>
- Haile, A., Joshi, B. K., Ayalew, W., Tegegne, A., Singh, A., & Yilm Z. (2008). Genetic evaluation of Ethiopian Boran cattle and their crosses with Holstein Friesian for milk constituent traits in central Ethiopia. *Journal of Cell and Animal Biology*, 2(10), 171-176.
- Hojman, D., Kroll, O., Adin, G., Gips, M., Hanochi, B., & Ezra E. (2004). Relationships between milk urea and production, nutrition and fertility traits in Israeli dairy herds. *Journal of Dairy Science*, 87(4), 1001-1011. [https://doi.org/10.3168/jds.S0022-0302\(04\)73245-2](https://doi.org/10.3168/jds.S0022-0302(04)73245-2)
- Horky, P. (2014). Effect of protein concentrate supplement on the qualitative and quantitative parameters of milk from dairy cows in organic farming. *Annals of Animal Science*, 14(2), 341-52. <https://doi.org/10.2478/aoas-2014-0008>
- Johnson, R. G., & Young, A. J. (2003). The association between milk urea nitrogen and DHI production variables in western commercial dairy herds. *Journal of Dairy Science*. 86(9), 3008–3015. [https://doi.org/10.3168/jds.S0022-0302\(03\)73899-5](https://doi.org/10.3168/jds.S0022-0302(03)73899-5)
- Kaygısız, A., & Şahin, O. (2023). Bazı sütçü sığır ırklarında somatik hücre sayısı (SHS) ve süt üre nitrojen (MUN) seviyesinin süt verimi ve bileşimine etkileri. *Mustafa Kemal University Journal of Agricultural Sciences*, 28(2), 290-307. <https://doi.org/10.37908/mkutbd.1220523>
- Kananub, S., Pechkerd, P., Van Leeuwen, J., Stryhnc. H., & Arunvipasa P. (2020). Evaluation of influence of milk urea nitrogen on reproductive performance in smallholder dairy farms. *Australian Veterinary Journal*, 98(8), 375-379. <https://doi.org/10.1111/avj.12946>
- Khastayeva, A. Z., Zhamurova, V. S., Mamayeva, L. A., Kozhabergenov, A. T., Karimov, N. Z., & Muratbekova, K. M. (2021). Qualitative indicators of milk of Simmental and Holstein cows in different seasons of lactation. *Veterinary World*, 14(4), 956-963. <https://doi.org/10.14202/vetworld.2021.956-963>
- Kitonjić, J. (2014). Sadržaj mliječne masti i proteina kao indikator nutritivnog statusa krava. Završni rad. Univerzitet u Banjoj Luci, Poljoprivredni fakultet.

- Konjačić, M., Kelava, N., Lukić, S., Ivanković, A., Prpić, Z., Vnućec, I., Ramljak, J., & Mijić P. (2010). Non-nutritional factors of milk urea concentration in Holstein cows from large dairy farms in Croatia. *Mljekarstvo*, 60(3), 166-174.
- Kirovski, D., Šamanc, H. & Prodanović, R. (2012). Procjena energetskeg statusa krava na osnovu koncentracije proteina i ureje u mlijeku. *Veterinarski glasnik*, 66 (1-2), 97-110.
- Kubesova, M., Fazon, T., Melich, J., Travnicek, J., & Marsalek, M. (2009). Analysis of milk urea and citrate content during the postpartal period and their impact on reproduction in dairy cows. *Výzkum v Chovu Skotu*, 51(1), 2-13.
- Lacková, P. T., Maskařová, I., Mihok, T., & Fraňová, B. (2024). Using milk urea to evaluate production, efficiency of nitrogen utilization and urinary nitrogen excretion during the lactation in dairy cows. *Folia Veterinaria*, 68(4), 19-24. <https://doi.org/10.2478/fv-2024-0033>
- Marenjak, T., Poljičak-Milas, N., & Stojević, Z. (2004): Svrha određivanja koncentracije ureje u kravljem mlijeku. *Praxis veterinaria*, 52(3), 233-241.
- Marenjak, T.S., & Poljicak-Milas, N. (2007): Seasonal variations in raw milk quality and milk production on smallholder dairy farms in Croatia. *Milchwissenschaft*. 62(3), 273-275.
- Olmos Colmenero, J. J., & Broderick, G. A. (2006). Effect of Dietary Crude Protein Concentration on Milk Production and Nitrogen Utilization in Lactating Dairy Cows. *Journal of Dairy Science*, 89(5), 1704-1712. [https://doi.org/10.3168/jds.S0022-0302\(06\)72238-X](https://doi.org/10.3168/jds.S0022-0302(06)72238-X)
- Oudah, E. Z. M. (2009). Non-genetic factors affecting somatic cell count, milk urea content, test-day milk yield and milk protein percent in dairy cattle of the Czech Republic using individual test-day records. *Livestock Research for Rural Development*, 21(5), 1-25. <https://doi.org/10.21608/jappmu.2009.119192>
- Rajala-Schultz, P. J., Saville, W. J. A., Frazer, G. S., & Wittum, T. E. (2001). Association between milk urea nitrogen and fertility in Ohio dairy cows. *Journal of Dairy Science*, 84(2), 482-489. [https://doi.org/10.3168/jds.s0022-0302\(01\)74498-0](https://doi.org/10.3168/jds.s0022-0302(01)74498-0)
- Roy, B., Brahma, B., Ghos, S., Pankaj, P. K., & Mandal G. 2011. Evolution of milk urea concentration as useful indicator for dairy herd management: A Review. *Asian Journal of Animal and Veterinary Advances*, 6(1), 1-19. <https://doi.org/10.3923/ajava.2011.1.19>
- Ruska, D., D. Jonkus, D., & Cielava, L. (2017). Monitoring of ammonium pollution from dairy cows farm according of urea content in milk. *Agronomy Research*, 15 (2), 553–564.
- Savić, Đ., Matarugić, D., Delić, N., Kasagić, D. & Stojanović, M. (2010). Određivanje organskih sastojaka mlijeka kao metoda ocjene energetskeg statusa mliječnih krava. *Veterinarski glasnik*, 64 (1-2), 21-32.
- Savić, Đ., Kasagić, D., Jotanović, S., Matarugić, D., Šarić, M. & Mijatović R. (2011). Procjena energetskeg statusa krava u ranoj laktaciji na osnovu određivanja organskih sastojaka mlijeka. *Agrozanje*, 67-74.
- Savić, Đ., Jotanović, S., Borojević, M., Vekić, M., & Čolović-Šarić, Z. (2019). Chemical composition of milk as an indicator of nutritional state of cows. *VIII International symposium on agricultural sciences AgroReS 2019, Book of Proceedings*, pp 84-95.
- Shulga, L.V., & Starovoytov, D. P. (2014). The influence of technology milking cows on the quality of milk. *Sci. Notes UO VGAVM*, 50(2), 342-345.
- Siatka, K., Sawa, A., Bogucki, M., & Krężel-Czopek, S. (2020). The effect of milk urea level on fertility parameters in Holstein–Friesian dairy cows. *Turkish Journal of Veterinary & Animal Sciences*, 44(1), 42-46. <https://doi.org/10.3906/vet-1810-41>
- Spiekers, H., & Obermaier, A. (2012). Milchharnstoffgehalt und N-Ausscheidung (Milk urea content and N excretion). Poing-Grub: *Institut für Tierernährung und Futterwirtschaft*. SuB Heft 4-5/07.
- Važić, B., Drinić, M., Kasagić, D., Popadić, S., & Rogić, B. (2020). Metabolic profile of the blood of Simmental cows during a production cycle. *Veterinarski Arhiv*, 90(1), 11-18. <https://doi.org/10.24099/vet.arhiv.0371>

- Uzun, N., & KOÇ, A. (2025). A Study on Milk Quality Characteristics of Simmental (Fleckvieh) Cows Reared Karacabey District of Bursa Province. *Journal of Animal Production*, 66 (1), 15-24. <https://10.29185/hayuretim.1581306>
- Šamanc, H., Kirovski, D., Dimitrijević, B., Vujanac, I., Damjanović, Z. & Polovina, M. (2006). Procjena energetskeg statusa krava u laktaciji određivanjem koncentracije organskih sastojaka mleka. *Veterinarski glasnik*, 60 (5-6), 283-297.
- Walstra, P., Wouters Jan, T. M., & Geurts, T. J. (Eds.). (2006). *Dairy Science and Technology* CRC Press; Taylor and Francis Group. <https://doi.org/10.1201/9781420028010>
- Zavadilová, L., Kasna, E., Krupova, Z., & Klimova, A. (2021). Health traits in current dairy cattle breeding: A review. *Czech Journal of Animal Science*, 66 (7), 235-250. <https://doi.org/10.17221/163/2020-CJAS>
- Young, A. (2001). Milk urea nitrogen (MUN). *Utah State University, Logan, Utah*.

Regional differences in milk production traits and mastitis prevalence in Holstein cows

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Abstract

The aim of this research was to analyse the variability of milk production traits and mastitis prevalence in Holstein cows across three breeding regions in Croatia: Central, Eastern, and Mediterranean. Data were derived from test-day records and included daily milk yield (DMY), fat and protein content, somatic cell count (SCC), and urea concentration. The differences due to the breeding region were observed in all analysed traits. The Eastern region exhibited the highest milk yield and fat production, which may be associated with more optimized nutritional and herd management practices. In contrast, the Mediterranean region recorded the lowest production parameters, likely reflecting the impact of environmental stressors, particularly elevated temperatures. The Central region showed intermediate production levels but the highest SCC values, indicating a greater risk of compromised udder health. Mastitis prevalence varied among regions, with the highest incidence of clinical mastitis recorded in the Central region. Similar regional patterns were observed for subclinical mastitis. These findings confirm the multifactorial influence of environmental conditions, management systems, and genetic background on milk production performance and udder health. In conclusion, the results highlight the importance of region-specific management strategies in dairy production systems. Identifying regional variability in milk traits and mastitis prevalence provides a basis for developing targeted interventions aimed at improving productivity, udder health, and overall animal welfare in Holstein populations.

Keywords: Holstein cows, milk production, mastitis prevalence, breeding region, somatic cell count

INTRODUCTION

Dairy cattle are susceptible to various health disorders that can adversely affect their milk production, well-being, and overall efficiency of dairy farms (Hogeveen et al., 2019). The most commonly reported disorders include mastitis, metabolic imbalances, lameness, digestive disturbances, reproductive dysfunctions, respiratory diseases, parasitic infections, and heat stress. Maintaining the health and productivity of dairy cattle requires the implementation of preventive measures, routine veterinary examinations, and appropriate treatments (Nir, 2003). Mastitis, an inflammatory disease of the mammary gland, is one of the most prevalent and economically significant health concerns in dairy farming (Seegers et al., 2003; Halasa et al., 2007; Benić et al., 2018; Hadrach et al., 2018; Maćešić et al., 2022; Chen et al., 2023). Therefore, mastitis represents a major challenge for the dairy industry, as it negatively impacts both individual animal performance and overall herd productivity (Halasa et al., 2007; Hadrach et al., 2018; Chen et al., 2023). The consequences of this disease encompass several key areas, including reduced milk yield, alterations in milk composition, economic losses, compromised milk quality, increased veterinary expenditures, reproductive inefficiencies, higher culling rates,

elevated somatic cell count (SCC), concerns regarding animal welfare, potential disease transmission within the herd, and long-term impairments to udder health (Halasa et al., 2007).

A decline in milk production is a direct consequence of mastitis, primarily due to the inflammatory response and structural damage to the mammary gland (Seegers et al., 2003). Additionally, infected cows often produce milk with compositional changes, including elevated SCC, increased concentrations of white blood cells, and imbalances in key milk constituents such as fat and protein (Schukken et al., 2003). The economic ramifications of mastitis are substantial, as financial losses stem from reduced milk output, veterinary interventions, discarded milk, and the need for culling chronically affected cows (Halasa et al., 2007; Hadrich et al., 2018). The costs associated with mastitis management are further exacerbated by veterinary treatments, including antibiotic therapy and supportive care. Reproductive performance may also be adversely affected, with mastitis contributing to prolonged intervals between estrous cycles, lower conception rates, and extended days open, largely due to the systemic inflammatory effects on fertility (Seegers et al., 2003). In cases of severe or recurrent mastitis, culling is often necessary to control disease transmission and mitigate economic losses. From an animal welfare perspective, mastitis induces pain and discomfort, reinforcing the necessity of effective management strategies and timely therapeutic interventions to reduce suffering. The contagious nature of certain mastitis-causing pathogens further underscores the importance of implementing biosecurity measures to prevent disease spread within the herd (Benić et al., 2018). Additionally, persistent or recurrent mastitis can have long-term repercussions on udder health, potentially leading to irreversible tissue damage and sustained reductions in milk production, even following treatment (Schukken et al., 2003).

Mastitis affects various breeds, with general characteristics remaining similar across breeds, although variations in pathogen prevalence may exist (Nóbrega and Langoni, 2011). Holstein cows, recognized for their high milk yield, may be more vulnerable to mastitis due to the increased physiological stress placed on their mammary glands. Furthermore, Holsteins are considered to be more susceptible to mastitis caused by environmental pathogens, such as *Escherichia coli* and environmental streptococci. According to Benić et al. (2018), bovine mastitis is predominantly of infectious origin, with approximately 150 microbial species identified as potential causative agents. Among them, *Staphylococcus aureus* is the most frequently isolated pathogen, posing a potential health risk to humans. The same authors also emphasize that the dairy cow's environment is heavily colonized by microbial communities, with pathogens typically entering the mammary gland through contaminated bedding or mud. Holstein cows affected by mastitis may exhibit clinical signs such as udder swelling, redness, and increased local temperature. Milk from infected quarters may display alterations in color and consistency. Additionally, due to their larger udders and longer teats compared to other dairy breeds, Holsteins are more prone to injuries and contamination during milking, increasing the risk of mastitis development.

The early detection of mastitis in dairy cattle is crucial for effective disease management and therapeutic intervention (Argaw, 2016). Various diagnostic techniques and technologies are available to identify mastitis at an early stage, thereby improving treatment outcomes (Narváez-Semanate et al., 2022). Some of the most widely used approaches for mastitis detection include clinical assessment, somatic cell count (SCC) evaluation, measurement of milk electrical conductivity, udder temperature monitoring, bacteriological culture, the California Mastitis Test, automated milk analyzers, machine milking parameters, rapid mastitis tests, teat-end condition monitoring, and automated health surveillance systems. Furthermore, SCC is a widely recognized marker of mastitis, given its strong correlation with inflammatory responses in the mammary gland (Schukken et al., 2003; Ivanov et al., 2016; Petzer et al., 2017). Blackburn (1966) reported that SCC levels increase progressively across successive lactations due to a higher presence of polymorphonuclear leukocytes (PMNs). However, fluctuations in SCC within a single lactation are associated with an increase in both PMNs and other somatic cells in milk. According to Smith et al. (2001), an SCC below 100,000 cells/ml is indicative of a healthy udder, whereas Mikó et al. (2016) suggested that normal milk typically contains SCC values ranging from 20,000 to 100,000 cells/ml. Elevated SCC levels are associated with milk production losses, leading to economic consequences for dairy farmers (Mikó et al., 2016; Hadrich et al., 2018).

The extent of milk yield reduction depends on SCC distribution at the individual cow level and across different parities within the herd (Chen et al., 2023). Pfützner and Ózsvári (2016) reported that cows with SCC values between 50,000 and 100,000 cells/ml experienced milk yield reductions exceeding 8%, while

those with SCC levels ranging from 100,000 to 250,000 cells/ml exhibited production losses exceeding 15%, reaching up to 18% in some cases. Gantner et al. (2011, 2017) investigated the relationship between heat stress and SCC in Holstein cows, highlighting fluctuations in SCC based on the temperature-humidity index, daily milk yield, breed, and parity. Furthermore, the effect of lactation stage, parity, season, and geographic region on SCC levels has been documented in multiple studies (Blackburn, 1966; Seegers et al., 2003; Nóbrega and Langoni, 2011; Yang et al., 2013; Tomazi et al., 2018; Weber et al., 2020; Stocco et al., 2023; Chen et al., 2023).

Considering the importance of mastitis prevalence analysis on dairy farms, this research aimed to determine the variability in the daily milk traits (milk quantity and quality) and the prevalence of subclinical and clinical mastitis in Holsteins due to the region of breeding using the test-day records.

MATERIAL AND METHODS

The statistical analysis in this research was based on test-day records of Holstein cows, collected over a 10-year period from January 2013 to December 2022 as part of routine milk recording. The milk recording procedure followed the standards established by the International Committee for Animal Recording (ICAR, 2017) for alternative milk recording methods (AT4/BT4). This approach involved measuring milk yield and obtaining milk samples from each cow during either the morning or evening milking at four-week intervals. Furthermore, data collection was conducted by trained officers from the Croatian Agency for Agriculture and Food (HAPIH) for the AT4 method, while farm employees trained in milk recording performed the BT4 method. Following sample collection, milk was transported to the accredited Central Laboratory for Milk Quality Control (SLKM) of HAPIH for analysis, ensuring compliance with ICAR (2017) protocols.

The chemical composition of milk, including fat, protein, lactose, total solids, solids-not-fat, urea, casein, free fatty acids, pH, ketone bodies, and freezing point, was analyzed using infrared spectrophotometry on MilkoScan analyzers. Additionally, somatic cell counts were determined using Fossomatic analyzers based on the fluoro-opto-electronic method.

Prior to the application of logical data control procedures, the dataset obtained from HAPIH comprised over 4 million test-day records. As part of the data validation process, records that did not conform to the standards established by ICAR (2017) were excluded. Additionally, records with incomplete or inaccurate information related to breed, farm identification, breeding region, lactation stage, parity, age at first calving, calving date, and milk recording date were removed to ensure data accuracy and consistency. Following these quality control measures, the final dataset included 2,509,222 test-day records of Holstein cows. Furthermore, regarding the location of farm records were grouped into three breeding regions: Central, Eastern, and Mediterranean.

Daily somatic cell count (SCC) was used as a key indicator for detecting subclinical and clinical mastitis in dairy cows. Cows were categorized into mastitis classes based on SCC thresholds: cows with SCC below 200,000 cells/ml were classified as healthy, those with SCC between 200,000 and 400,000 cells/ml were considered to have subclinical mastitis, while SCC exceeding 400,000 cells/ml indicated clinical mastitis.

The prevalence of subclinical and clinical mastitis was determined as the proportion of cows within each mastitis category relative to the total number of animals, both overall and per breeding region.

Variability in milk production traits and SCC was assessed using descriptive statistics, including coefficients of variation, while frequency distributions were analyzed to evaluate the occurrence of mastitis categories across breeding regions. All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA), version 9.4.

RESULTS AND DISCUSSION

The results of the analysis of the variability in milk production traits of Holstein cows across different breeding regions are presented in the following table 1.

Table 1. Parameters of variability of daily milk yield traits of Holstein cows depending on the breeding region (Central, Eastern and Mediterranean Croatia)

Region	DMY kg				DFC %				DFY			
	N	Mean	SD	CV	N	Mean	SD	CV	N	Mean	SD	CV
CE	953964	23.3	9.32	39.917	953964	4.1	0.94	22.774	953964	1.0	0.43	44.854
ES	1440575	27.3	10.43	38.162	1440575	4.2	0.99	23.724	1440575	1.1	0.50	44.098
ME	114683	24.1	9.46	39.284	114683	3.9	0.90	23.131	114683	0.9	0.40	43.489
Total	2509222	25.7	10.17	39.605	2509222	4.1	0.97	23.379	2509222	1.1	0.47	45.193

Region	DPC %				DPY				DLC %			
	N	Mean	SD	CV	N	Mean	SD	CV	N	Mean	SD	CV
CE	953964	3.4	0.44	12.995	953964	0.8	0.30	38.747	953964	4.5	0.22	4.899
ES	1440575	3.4	0.42	12.134	1440575	0.9	0.33	35.807	1440575	4.5	0.23	5.060
ME	114683	3.4	0.43	12.830	114683	0.8	0.30	37.593	114683	4.5	0.22	4.982
Total	2509222	3.4	0.43	12.506	2509222	0.9	0.33	37.757	2509222	4.5	0.22	4.999

Region	SSC*10 ³				SSC log				Urea mg/dL			
	N	Mean	SD	CV	N	Mean	SD	CV	N	Mean	SD	CV
CE	953619	496.7	1102.60	221.981	953619	7.3	2.08	28.431	953964	20.1	9.71	48.298
ES	1440364	416.6	1018.35	244.427	1440364	7.0	2.06	29.517	1440575	23.5	8.36	35.653
ME	114677	397.1	935.09	235.473	114677	6.9	2.10	30.362	114683	23.0	9.64	41.905
Total	2508660	446.2	1048.38	234.970	2508660	7.1	2.08	29.225	2509222	22.2	9.10	41.071

*DMY – daily milk yield (kg); DFC – daily fat content (%); DFY – daily fat yield (kg); DPC – daily protein content (%); daily protein yield (kg); DLC – daily lactose content (%); SSC*10³ – somatic cell count; SSC log – log value of SSC; Urea – urea content in milk (mg/dL); CE – Central; ES – Eastern; ME – Mediterranean

Regarding daily milk yield (DMY), the highest average value was observed in the Eastern region (ES) with 27.3 kg, followed by the Mediterranean region (ME) at 24.1 kg and the Central region (CE) at 23.3 kg. The coefficient of variation (CV) for DMY was relatively similar across all regions, ranging from 38.162% in the Eastern region to 39.917% in the Central region, indicating moderate variability in milk yield within each region. For daily fat content (DFC), cows from the Eastern region (ES) had the highest average fat percentage (4.2%), followed closely by the Central region (CE) at 4.1%, while cows in the Mediterranean region (ME) had the lowest fat content (3.9%). The CV values for fat content ranged from 22.774% in the Central region to 23.724% in the Eastern region, demonstrating moderate variability in fat content across regions. In terms of daily fat yield (DFY), the Eastern region (ES) also exhibited the highest average fat yield at 1.1 kg, while the Central region (CE) and Mediterranean region (ME) had lower values of 1.0 kg and 0.9 kg, respectively. The CV for DFY ranged from 43.489% in the Mediterranean region to 45.193% in the overall dataset, indicating a considerable amount of variation in fat yield. For the percentage of protein (DPC), the averages were similar across regions, with values of 3.4% in all three regions (CE, ES, ME). The CV for DPC ranged from 12.134% in the Eastern region to 12.995% in the Central region, indicating relatively low variability in dry matter content among cows in each region. The average daily protein yield (DPY) was highest in the Eastern region (ES) at 0.9 kg, followed by the Central (CE) and Mediterranean (ME) regions, both with 0.8 kg. The CV values for DPY ranged from 35.807% in the Eastern region to 38.747% in the Central region, indicating considerable variability in protein yield within each region. For daily lactose (DLC), cows in all regions showed similar values, with averages of 4.5%. The CV for DLC was low, ranging from 4.899% in the Central region to 5.060% in the Eastern region, suggesting minimal variability in daily lactose content across regions.

The somatic cell count (SSC) was highest in the Central region (CE), with an average of 496.7×10^3 cells, followed by the Eastern (ES) and Mediterranean (ME) regions at 416.6×10^3 cells and 397.1×10^3 cells, respectively. The CV for SSC ranged from 221.981% in the Central region to 244.427% in the

Eastern region, indicating significant variability in somatic cell count within each region. The log-transformed SSC (SSC log) was relatively consistent across regions, with the Central region (CE) having an average of 7.3, the Eastern region (ES) 7.0, and the Mediterranean region (ME) 6.9. The CV values for SSC log were comparable, ranging from 28.431% in the Central region to 30.362% in the Mediterranean region. Regarding urea content, cows from the Eastern region (ES) had the highest average urea level at 23.5 mg/dL, followed by the Mediterranean region (ME) at 23.0 mg/dL and the Central region (CE) at 20.1 mg/dL. The CV for urea content was highest in the Central region (48.298%), followed by the Mediterranean region (41.905%) and the Eastern region (35.653%).

The obtained results indicate clear regional differences in milk production parameters among Holstein cows, highlighting the influence of environmental, management, and feeding factors. The Eastern region generally exhibited the highest values for milk yield (DMY), fat content, and fat yield (DFY), likely reflecting more efficient milk production due to superior management practices, optimized feeding strategies, and potentially favorable climatic conditions (Gantner et al., 2011; Gantner et al., 2017). In contrast, the Mediterranean region recorded the lowest values, which can be attributed to challenging environmental conditions, particularly elevated temperatures, negatively affecting cow performance and milk quality (Gantner et al., 2011; Yang et al., 2013; Gantner et al., 2017). The Central region showed intermediate results across most parameters, although it presented the highest somatic cell count (SCC), indicating potential udder health concerns such as mastitis (Schukken et al., 2003; Cvetnić et al., 2016). Urea levels were highest in the Eastern region, suggesting higher protein intake or differences in nitrogen metabolism. The coefficient of variability for many traits ranged from moderate to high, demonstrating notable differences in milk production characteristics among individual cows within each region. Overall, these findings underscore the impact of regional factors on milk production and emphasize the need for tailored management strategies aimed at improving milk yield, enhancing milk quality, and maintaining udder health under differing environmental and management conditions (Antanaitis et al., 2021).

The results presented in Table 2 show the prevalence of mastitis in Holstein cows across three different breeding regions in Croatia: Central, Eastern, and Mediterranean.

Table 2. Mastitis prevalence in Holstein cows depending on the breeding region (Central, Eastern and Mediterranean Croatia)

Region		Animal's health status			Total
		Clinical	Normal	Subclinical	
Central	No	240930	562413	150621	953964
	%	25.26	58.96	15.79	100.00
Eastern	No	290634	954010	195931	1440575
	%	20.17	66.22	13.60	100.00
Mediterranean	No	23651	75848	15184	114683
	%	20.62	66.14	13.24	100.00

In the Central region, the prevalence of clinical mastitis was 25.26%, which represents the highest proportion of mastitis cases among the three regions. The normal (healthy) cows in this region accounted for 58.96%, while subclinical mastitis affected 15.79% of the cows. This suggests a relatively higher prevalence of clinical mastitis in the Central region compared to the other regions, which may be influenced by regional management practices, environmental conditions, or other factors that affect udder health. The Eastern region showed a lower prevalence of clinical mastitis at 20.17%, and a higher proportion of cows were classified as healthy, with 66.22% of cows in the normal group. Subclinical mastitis was present in 13.6% of the cows, which is similar to the Central region. The lower prevalence of clinical mastitis in the Eastern region may reflect better udder health management or more favorable environmental conditions for cow health. In the Mediterranean region, the prevalence of clinical mastitis was the lowest among the three regions, at 20.62%, and the proportion of healthy cows was also lower compared to the other two regions, at 66.14%. Subclinical mastitis affected 13.24% of the cows, which is slightly lower than in the Central and Eastern regions. The lower prevalence of clinical mastitis in the Mediterranean region may be linked to different environmental challenges, such as higher temperatures and humidity, which could potentially reduce the overall prevalence of clinical mastitis or affect the way it is diagnosed and managed. Finally, the Central region had the highest prevalence of clinical mastitis,

while the Eastern region had a higher proportion of healthy cows, and the Mediterranean region had the lowest prevalence of clinical mastitis. These regional differences suggest that environmental, management, and possibly genetic factors play a role in the prevalence and management of mastitis. It highlights the importance of region-specific strategies for mastitis control, including improving udder health management, optimizing cow comfort, and addressing any regional environmental challenges that may affect milk production and quality.

The results in Table 3 present the variability of daily milk yield traits of Holstein cows in relation to their health status, including healthy, subclinical mastitis, and clinical mastitis classes.

Table 3. Parameters of variability of daily milk yield traits of Holstein cows depending on the health status (health, subclinical or clinical mastitis)

Mastitis class	DMY kg				DFC %				DFY			
	N	Mean	SD	CV	N	Mean	SD	CV	N	Mean	SD	CV
Clinical	555215	23.6	10.09	42.69	555215	4.2	0.99	23.55	555215	1.0	0.47	48.26
Normal	1592271	26.8	10.06	37.50	1592271	4.1	0.96	23.42	1592271	1.1	0.47	43.51
Subclinical	361736	23.8	10.00	42.10	361736	4.2	0.96	22.76	361736	1.0	0.46	46.73
Total	2509222	25.7	10.17	39.61	2509222	4.1	0.97	23.38	2509222	1.1	0.47	45.19
Mastitis class	DPC %				DPY				DLC %			
	N	Mean	SD	CV	N	Mean	SD	CV	N	Mean	SD	CV
Clinical	555215	3.5	0.44	12.72	555215	0.8	0.33	40.75	555215	4.4	0.26	5.90
Normal	1592271	3.4	0.42	12.30	1592271	0.9	0.32	35.87	1592271	4.5	0.19	4.215
Subclinical	361736	3.5	0.44	12.60	361736	0.8	0.33	40.09	361736	4.4	0.22	4.99
Total	2509222	3.4	0.43	12.51	2509222	0.9	0.33	37.76	2509222	4.5	0.22	4.99
Mastitis class	SSC *10 ³				SSC log				Urea			
	N	Mean	SD	CV	N	Mean	SD	CV	N	Mean	SD	CV
Clinical	555215	1618.5	1780.17	109.99	555215	10.1	1.14	11.31	555215	21.3	9.49	44.57
Normal	1591709	74.6	50.74	67.99	1591709	5.8	1.14	19.54	1592271	22.6	8.91	39.43
Subclinical	361736	281.7	56.86	20.18	361736	8.1	0.29	3.56	361736	21.6	9.20	42.64
Total	2508660	446.2	1048.38	234.97	2508660	7.1	2.08	29.23	2509222	22.2	9.10	41.07

DMY – daily milk yield (kg); DFC – daily fat content (%); DFY – daily fat yield (kg); DPC – daily protein content (%); daily protein yield (kg); DLC – daily lactose content (%); SSC*10³ – somatic cell count; SSC log – log value of SSC; Urea – urea content in milk (mg/dL)

Cows with clinical mastitis had a slightly lower mean daily milk yield (DMY) of 23.6 kg compared to the healthy group, which had a mean DMY of 26.8 kg. The subclinical group showed a mean DMY of 23.8 kg, which is close to the clinical group but still lower than the normal group. Despite this, the coefficients of variation (CV) for DMY were similar across all mastitis classes, ranging from 37.5% in the healthy group to 42.7% in the clinical group, suggesting considerable variation in milk yield within each health status category.

Furthermore, in terms of daily fat content (DFC %), the clinical mastitis group had a slightly higher mean value (4.2%) compared to the healthy group (4.1%) and the subclinical group (4.2%), but the variability (SD) was similar across groups. The CV for DFC % was highest in the clinical group (23.6%), indicating more variation in fat content within the clinical mastitis group compared to the other two groups. Daily fat yield (DFY) was slightly higher in the healthy cows (1.1 kg) compared to both the clinical (1.0 kg) and subclinical groups (1.0 kg). The variability in DFY, was similar across all groups, indicating that the difference in daily fat yield between the groups may not be substantial, although there is still variability within each group. In terms of daily protein content (DPC), cows with clinical and subclinical mastitis showed similar values, with a mean of 3.5%, while normal cows had slightly lower value at 3.4%. Regarding daily protein yield (DPY), clinical and subclinical cows had a mean of 0.8 kg, with high

variability (CV of 40.75% and 40.09%, respectively), while normal cows had a higher mean of 0.9 kg and lower variability (CV of 35.87%). Daily lactose content (DLC%) was relatively consistent across all groups, with clinical and subclinical cows having a mean of 4.4% and normal cows 4.5%. The low variability in lactose content (CV around 5%) across all groups suggests that mastitis, even in subclinical cases, does not significantly alter lactose production (Antanaitis et al., 2021). For somatic cell count (SCC $\times 10^3$), the clinical mastitis group had the highest mean (1618.5×10^3), which is expected given the direct correlation between high SCC and mastitis (Petzer et al., 2017). The healthy cows had a much lower mean SCC (74.6×10^3), while the subclinical mastitis group had an intermediate mean (281.7×10^3). The CV for SCC was also highest in the clinical group (109.99), indicating more variability in SCC within this group compared to the other two. Urea levels were relatively similar across the groups, with the healthy cows having a slightly higher mean (22.6 mg/dL) compared to the clinical (21.3 mg/dL) and subclinical (21.6 mg/dL) groups. The CV for urea levels was relatively consistent across all groups, suggesting that urea levels are not heavily influenced by mastitis severity. The differences in analysed traits emphasize the impact of mastitis, particularly clinical mastitis, on milk production. The higher SCC and lower milk yield in cows with clinical mastitis were expected and suggest a direct negative effect on udder health and milk production. However, cows with subclinical mastitis did not show a significant reduction in milk yield or fat content, but the increased SCC still indicates an ongoing subclinical infection that could affect herd performance in the long term.

CONCLUSION

The obtained results indicate notable regional variations in milk production traits and the prevalence of mastitis in Holstein cows. The Eastern region demonstrated the highest levels of milk yield, fat content, and fat yield, suggesting a more efficient approach to milk production compared to the Central and Mediterranean regions. Moreover, the Central region showed elevated somatic cell counts (SCC), which may signal potential udder health issues. The prevalence of both clinical and subclinical mastitis was notably highest in the Central region. These results emphasize the significance of regional factors in the variability of milk yield, quality, and udder health. Consequently, sustainable management strategies are necessary to enhance milk production and quality while effectively addressing mastitis and mitigating its impact on dairy farms. Finally, research results emphasised the importance of considering regional differences to optimize the health and production of dairy cattle.

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REFERENCES

- Antanaitis, R., Juozaitienė, V., Jonike, V., Baumgartner, W., & Paulauskas, A. (2021). Milk lactose as a biomarker of subclinical mastitis in dairy cows. *Animals*, 11, 1736. <https://doi.org/10.3390/ani11061736>
- Argaw, A. (2016). Review on epidemiology of clinical and subclinical mastitis on dairy cows. *Food Science and Quality Management*, 52, 56–65.
- Benić, M., Maćešić, N., Cvetnić, L., Habrun, B., Cvetnić, Ž., Turk, R., Đuričić, D., Lojkić, M., Dobranić, V., Valpotić, H., Grizelj, J., Gračner, D., Grbavac, J., & Samardžija, M. (2018). Bovine mastitis: a persistent and evolving problem requiring novel approaches for its control – a review. *Veterinarski arhiv*, 88, 535–557. <https://doi.org/10.24099/vet.arhiv.0116>
- Blackburn, P. S. (1966). The variation in the cell count of cow's milk throughout lactation and from one lactation to the next. *Journal of Dairy Research*, 33, 193–198. <https://doi.org/10.1017/S0022029900011857>
- Chen, S., Zhang, H., Zhai, J., Wang, H., Chen, X., & Qi, Y. (2023). Prevalence of clinical mastitis and its associated risk factors among dairy cattle in mainland China during 1982–2022: a systematic

- review and meta-analysis. *Frontiers in Veterinary Science*, 10, 1185995. <https://doi.org/10.3389/fvets.2023.1185995>
- Cvetnić, L., Samardžija, M., Habrun, B., Kompes, G., & Benić, M. (2016). Microbiological monitoring of mastitis pathogens in the control of udder health in dairy cows. *Slovenian Veterinary Research*, 53, 131–140.
- Gantner, V., Bobic, T., Gantner, R., Gregic, M., Kuterovac, K., Novakovic, J., & Potocnik, K. (2017). Differences in response to heat stress due to production level and breed of dairy cows. *International Journal of Biometeorology*, 61, 1675–1685. <https://doi.org/10.1007/s00484-017-1348-7>
- Gantner, V., Mijić, P., Kuterovac, K., Solić, D., & Gantner, R. (2011). Temperature-humidity index values and their significance on the daily production of dairy cattle. *Mljekarstvo*, 61, 56–63.
- Hadrich, J. C., Wolf, C. A., Lombard, J., & Dolak, T. M. (2018). Estimating milk yield and value losses from increased somatic cell count on US dairy farms. *Journal of Dairy Science*, 101, 3588–3596. <https://doi.org/10.3168/jds.2017-13840>
- Halasa, T., Huijps, K., Østerås, O., & Hogeveen, H. (2007). Economic effects of bovine mastitis and mastitis management: A review. *Veterinary Quarterly*, 29, 18–31. <https://doi.org/10.1080/01652176.2007.9695224>
- Hogeveen, H., Steeneveld, W., & Wolf, C. A. (2019). Production diseases reduce the efficiency of dairy production: A review of the results, methods, and approaches regarding the economics of mastitis. *Annual Review of Resource Economics*, 11, 289–312. <https://doi.org/10.1146/annurev-resource-100518-093954>
- ICAR – International Committee for Animal Recording (2017). Section 2 - Guidelines for Dairy Cattle Milk Recording.
- Ivanov, G. Y., Bilgucu, E., Ivanova, I. V., Uzatici, A., & Balabanova, T. B. (2016). Monitoring of the somatic cells count for improving milk and dairy products quality. *Scientific Works of University of Food Technologies*, 63, 90–97.
- Maćešić, N., Bačić, N., Bačić, G., Lojkić, M., Samardžija, M., Benić, M., Prvanović Babić, N., Butković, I., Šavorić, J., Efendić, M., & Karadjole, T. (2022). Selective dry cow treatment. *Veterinarska stanica*, 53, 735–743. <https://doi.org/10.46419/vs.53.6.8>
- Mikó, E., Atasever, S., Gráff, M., & Erdem, H. (2016). Influence of somatic cell count on daily milk yield and milk production losses in primiparous Hungarian Holstein cows. In *In Memoriam Ferenc Kovács International Congress on Veterinary and Animal Science*, Budapest, Hungary
- Narváez-Semanate, J. L., Daza-Bolaños, C. A., Valencia-Hoyos, C. E., Hurtado-Garzón, D. T., & Acosta-Jurado, D. C. (2022). Diagnostic methods of subclinical mastitis in bovine milk: an overview. *Revista Facultad Nacional de Agronomía Medellín*, 75, 1–12. <https://doi.org/10.15446/rfnam.v75n3.100520>
- Nir, O. (2003). What are production diseases, and how do we manage them? *Acta Veterinaria Scandinavica*, 44, 21–32. <https://doi.org/10.1186/1751-0147-44-S1-S21>
- Nóbrega, D. B., & Langoni, H. (2011). Breed and season influence on milk quality parameters and in mastitis occurrence. *Pesquisa Veterinária Brasileira*, 31, 1045–1052. <https://doi.org/10.1590/S0100-736X2011001200002>
- Petzer, I. M., Karzis, J., Donkin, E. F., Webb, E. C., & Etter, E. M. C. (2017). Validity of somatic cell count as indicator of pathogen-specific intramammary infections. *Journal of the South African Veterinary Association*, 88(0), a1465. <https://doi.org/10.4102/jsava.v88i0.1465>
- Pfützner, M., & Ózsvári, L. (2016). The economic impact of decreased milk production due to subclinical mastitis in East German dairy herds. In *Proceedings of the 29th World Buiatrics Congress*, Dublin, Ireland
- Schukken, Y. H., Wilson, D. J., Welcome, F., Garrison-Tikofsky, L., & Gonzalez, R. N. (2003). Monitoring udder health and milk quality using somatic cell counts. *Veterinary Research*, 34, 579–596. <https://doi.org/10.1051/vetres:2003028>

- Seegers, H., Fourichon, C., & Beaudeau, F. (2003). Production effects related to mastitis and mastitis economics in dairy cattle herds. *Veterinary Research*, 34, 475–491. <https://doi.org/10.1051/vetres:2003027>
- Smith, K. L., Hillerton, J. E., & Harmon, R. J. (2001). Guidelines on normal and abnormal raw milk based on somatic cell counts and signs of clinical mastitis. *National Mastitis Council*, 9, 11–13.
- Stocco, G., Cipolat-Gotet, C., Stefanon, B., Zecconi, A., Francescutti, M., Mountricha, M., & Summer, A. (2023). Herd and animal factors affect the variability of total and differential somatic cell count in bovine milk. *Journal of Animal Science*, 101, skac406. <https://doi.org/10.1093/jas/skac406>
- Tomazi, T., Ferreira, G. C., Orsi, A. M., Gonçalves, J. L., Ospina, P. A., Nydam, D. V., Moroni, P., & Dos Santos, M. V. (2018). Association of herd-level risk factors and incidence rate of clinical mastitis in 20 Brazilian dairy herds. *Preventive Veterinary Medicine*, 161, 9–18. <https://doi.org/10.1016/j.prevetmed.2018.10.007>
- Weber, C. T., Corrêa Schneider, C. L., Busanello, M., Bandeira Calgaro, J. L., Fioresi, C. R., Gehrke, C. R., Da Conceição, J. M., & Haygert-Velho, I. M. P. (2020). Season effects on the composition of milk produced by a Holstein herd managed under semi-confinement followed by compost bedded dairy barn management. *Semina: Ciências Agrárias*, 41, 1667–1678. <https://doi.org/10.5433/1679-0359.2020v41n5p1667>
- Yang, L., Yang, Q., Yi, M., Pang, Z. H., & Xiong, B. H. (2013). Effects of seasonal change and parity on raw milk composition and related indices in Chinese Holstein cows in northern China. *Journal of Dairy Science*, 96, 6863–6869. <https://doi.org/10.3168/jds.2013-6846>

A comparative study of production performance of *Zophobas morio* and *Tenebrio molitor* as alternative protein sources in animal diets

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Abstract

Insects are increasingly recognized as a potential protein source for both animal and human consumption. In this study, two species, *Tenebrio molitor* and *Zophobas morio*, were compared, starting from the same initial mass until *T. molitor* began pupating and *Z. morio* stopped growing. Production parameters, individual growth, and total insect biomass were analyzed. Results showed that the larger species, *Zophobas morio*, was more efficient in terms of growth rate over time, while the smaller species, *T. molitor*, was more efficient in terms of feed utilization. *Zophobas* growth was 22% higher per week (4.41 g/week vs 3.62 g/week), whereas *Tenebrio* used 8% less feed to produce the same amount of larval mass. Moreover, the economic efficiency of production was more favorable for *T. molitor*. These findings indicate that species selection should depend on production goals: *T. molitor* is preferable for higher overall productivity and lower feed costs, while *Z. morio* may be chosen when larger individual insects are desired. The choice of insect species for production should be guided by the intended production goal.

Keywords: *Zophobas morio*, *Tenebrio molitor*, feed, growth efficiency, biomass yield

INTRODUCTION

It is assumed that with the growth of the human population, as indicated by various projections (Gerland et al. 2014), food will become an increasingly critical resource. The limited availability of agricultural land, public pressure toward greener solutions, and the increasingly pronounced effects of climate change have brought insects into the spotlight as a potential solution (Moruzzo et al., 2021; Bridge 2023; Aguilar-toalá and Vidal-limón 2025). Insects are gaining global attention as an alternative protein source for both human and animal consumption (Veldkamp et al. 2012). In fact, EU legislation permits the use of four insect species for food (EFSA, 2021; EFSA, 2022; EFSA, 2022b; EFSA, 2023).

The situation in Serbia is somewhat different. According to current regulations, insects produced locally in Serbia may be used as animal feed. Eight insect species are currently authorized (Official Gazzette, 2023). However, imported insect-based protein feed has already been present on the Serbian market for several years. Among insects studied as alternative protein sources, *Tenebrio molitor* is by far the most investigated species (Fasce et al. 2022; Pascual et al. 2024; First et al. 2024; Sprangers et al. 2024). There is substantial data available on its production characteristics, various rearing conditions, and density parameters, and production technology is relatively well established.

Zophobas species including *Zophobas morio*, although under investigation (Gomes Da Costa Lima et al. 2024; Marina Gourgouta et al. 2024; M. Gourgouta et al. 2024), are much less represented, primarily because they are not among the insect species authorized for human or animal consumption in EU countries. A large part of current research regarding these superworms focuses on their ability to degrade certain types of plastic materials, particularly polystyrene and other polymers (Miravalle et al. 2024; Sadat et al. 2024). This does not necessarily imply that they will not be used in the future as human or animal food, but research focus has predominantly been on species that present certain advantages compared to *Zophobas*.

There are conflicting opinions regarding the suitability of these two insect species as alternative protein sources in animal nutrition (Harsányi et al. 2020; Dragojlović et al. 2022). When considering their application, attention is not focused solely on the chemical composition of the obtained product, but also on production cyclicity and the possibility of achieving the highest possible yield, that is, the quantity of insects intended for further use.

It is commonly believed that *Tenebrio molitor* is more favorable for rearing due to its shorter production cycle, easier management, and lower sensitivity. However, comparisons of production parameters between these two species are often insufficiently precise, given that their production cycles differ significantly.

The aim of this study was to conduct a comparative analysis of these insects, with particular emphasis on production parameters such as weight gain, feed conversion, final mass per replicate, and individual body mass. Ultimately, all results were evaluated from an economic perspective, focusing on the cost of production.

MATERIAL AND METHODS

Insects

The larvae of *Tenebrio molitor* and *Zophobas morio* used in this study originated from the Faculty of Agriculture, University of Novi Sad, Serbia. *Tenebrio molitor* larvae were reared under standard controlled conditions at 27°C and 60% relative humidity on a substrate composed of wheat bran (95%) and dried brewer's yeast (5%).

Ten days after adult emergence, adults were transferred to fresh substrate in standard rearing trays (50 × 60 × 10 cm) and allowed to mate and oviposit for 48 h, after which the adults were removed. The trays were then incubated in a climate-controlled chamber at 26 ± 1°C and 65 ± 5% relative humidity.

Seven days after oviposition, newly hatched larvae were supplemented with fresh carrot pieces every two days. Larvae were reared for 42 days, starting from the first observation of neonates. At the end of the pre-experimental rearing period, larvae were separated from residual substrate and frass using vertical sieves (0.75–1.25 mm mesh size) and transferred to clean trays, where they were starved for 24 h to allow gut clearance prior to experimentation. *Zophobas morio* larvae were reared in a similar manner to *T. molitor*, under identical environmental conditions and feeding regimes.

Diets

For the purpose of this study, both insect species were maintained on an identical diet of wheat bran, the detailed composition of which is presented in Table 1.

Table 1. Chemical composition of wheat bran used for feeding both insect species

	Dry matter	Crude protein	Ash	Ether extract	NDF	ADF
Wheat Bran	90.88	16.73	5.02	4.1	36.62	11.6

Experimental design

The initial total mass of insects was 2 grams for both *Tenebrio molitor* and *Zophobas* species. The experiment was conducted as a typical randomized design with four replicates, and all insects were provided the same diet. The larvae were approximately 28 days old, with an average individual mass of 5.36 mg for *T. molitor* and 28.01 mg for *Zophobas*.

Due to differences in species-specific environmental requirements, *T. molitor* larvae were maintained in an incubator at 26°C and 60% relative humidity, while *Zophobas* larvae were kept at a slightly higher temperature of 28°C and the same humidity. Feeding was provided *ad libitum*, based on visual inspection to ensure that food had been consumed, and water was supplied using agar.

For *T. molitor*, the experiment concluded when individuals began pupating. In the case of *Zophobas*, the experiment ended when two consecutive measurements of total mass showed no increase, indicating that growth had ceased.

Used formula

Feed Conversion Ratio (FCR, g/g) = Diet intake, g / (larval biomass at the end, g-larval biomass at the start, g)

Specific growth rate (SGR)%/day = ((lnFinal Bod weight – lnInitial body weight)/day3))*100

Statistics

Statistical analysis was performed using Statistica 14.1.0.8 (TIBCO Software Inc.). Data was first checked for homoscedascity, and after significant Leven's test, Welch t-test was applied. Differences were considered significant at $p < 0.05$. Feed cost per unit of gain was calculated using wheat bran price from the local Serbian market in February 2026.

RESULTS AND DISCUSSION

Table 2 presents the production parameters measured at the end of the experimental period. As shown, statistically significant differences were observed for all parameters, which are also evident from the raw data even without formal statistical analysis. The initial insect mass was 2 g, and the final mass differed by 17 g, clearly indicating a substantial difference. It should be noted that the experiment with *Tenebrio molitor* ended upon the appearance of the first pupae, 32 days after the start of the trial, whereas for *Zophobas morio*, the experiment concluded when insect mass stabilized, eight weeks from the start, resulting in an age difference between the two groups.

Table 2. Production parameters of two different insect species fed wheat bran

Production parameter	<i>Tenebrio molitor</i>	<i>Zophobas morio</i>	P value
Weight per tray, initial, g	2.00	2.00	
Growing period until pupation, weeks	5	8	
Weight per tray, final, g	20.1 ± 0.36	37.3 ± 1.30	p = 0.000
Weight gain per tray, g	18.1 ± 0.36	35.3 ± 1.30	p = 0.000
Weight gain per tray, per week, g	3.62 ± 0.07	4.41 ± 0.16	p = 0.001
Individual weight, initail, mg	5.36	28.01	
Individual weight, final, mg	70 ± 3.2	604 ± 12.7	p = 0.000
Individual weight gain, mg	65 ± 3.2	576 ± 12.7	p = 0.000
Individual weight gain per week, mg	13.0 ± 0.64	72.0 ± 1.59	p = 0.000
Specific growth rate, %/day	8.05 ± 0.14	5.48 ± 0.04	p = 0.000
Feed consumption per tray, g	42.5 ± 0.14	89.7 ± 0.57	p = 0.000
Feed conversion ratio, g/g	2.35 ± 0.05	2.55 ± 0.09	p = 0.012
Efficiency of conversion of ingested feed, g/100g	42.6 ± 0.90	39.3 ± 1.34	p = 0.003
Feed cost per unit of gain, EUR/kg	0.53	0.58	

Although the difference in feed conversion was relatively small (2.35 for *T. molitor* vs. 2.55 for *Z. morio*), it was statistically significant, confirming that *Tenebrio* utilizes feed more efficiently than *Zophobas* under the tested conditions. Specific growth of insects is expressed as the percentage change in body mass relative to the initial mass, enabling direct comparison between species with differing initial masses and experimental durations. Under the experimental conditions, *Tenebrio molitor* achieved a notably higher

specific growth (8.05%) than *Zophobas morio* (5.15%), demonstrating superior growth performance and highlighting *Tenebrio* as the more efficient species in terms of biomass gain.

Larger insect species, *Zophobas morio*, was more efficient considering needed time, while smaller species, *Tenebrio molitor*, was more efficient considering feed used. *Zophobas* growth was 22% larger in the same unit of time (4.41 g/week vs 3.62 g/week), while *Tenebrio* used 8% less feed to produce the same amount of larval mass. Estimating economic impact of difference in the feed usage is straightforward. Contrary to that, effects of faster growth are more complex and probably include reduced equipment, energy and labor costs, yet, growing densities also must be taken into account in this consideration.

Comparison of results across different studies is challenging due to substantial methodological differences. Dragojlović et al. 2022 concluded that mass yield in *Tenebrio molitor* was higher compared to *Zophobas morio*. In our study, however, the total mass yield at the end of the experiment was higher in *Zophobas morio*, since the trial for this species lasted until the completion of larval growth, which was nearly four weeks longer than for *Tenebrio molitor*. Although the weight gain expressed in grams per unit time was higher in *Zophobas*, *Tenebrio molitor* demonstrated better feed conversion efficiency, as confirmed by our results and faster growth in time.

Tenebrio molitor has been extensively studied in recent years, with reported results varying considerably (Biasato et al. 2017; Montalbán et al. 2023; Pascual et al. 2024; López-Gámez et al. 2024), primarily depending on the type of diet used, as numerous feeding trials have been conducted. Key production parameters, such as feed conversion ratio (FCR) when wheat bran was used, generally fall within the ranges reported in the literature, as does the specific growth rate (SGR).

Our results indicate that the choice of species depends on the production goal. If the objective is to achieve a higher individual final weight, *Zophobas morio* appears more suitable. However, if the goal is higher overall productivity and greater total biomass, *Tenebrio molitor* would be the preferred option.

CONCLUSION

The choice of insect species for production should be guided by the intended production goal. If the objective is to achieve higher individual growth and longer production cycles, *Zophobas morio* may be more suitable. Conversely, if the goal is maximized overall biomass production and more frequent production cycles, *Tenebrio molitor* appears to be the better option. Overall, the results suggest that species selection should align with production priorities, and under the conditions of this study, *Tenebrio molitor* offers a more efficient option for biomass gain, whereas *Zophobas morio* may be preferred when larger individual insects are desired.

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REFERENCES

- Aguilar-Toalá, J. E., & Vidal-Limón, A. M. (2025). Advancing food security with farmed edible insects: economic, social, and environmental aspects. *Insects*, 16(67), 1–15. <https://doi.org/10.3390/insects16010067>
- Biasato, I., Gasco, L., De Marco, M., Renna, M., Rotolo, L., Dabbou, S., Capucchio, M. T. et al. (2017). Effects of yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for female broiler chickens: implications for animal health and gut histology. *Animal Feed Science and Technology*, 234, 253–263. <https://doi.org/10.1016/j.anifeedsci.2017.09.014>

- Data Bridge (2023). Europe insect protein market – industry trends and forecast to 2030. Data Bridge Market Research.
- Dragojlović, D., Đuragić, O., Pezo, L., Popović, Lj., Rakita, S., & Tomičić, Z. (2022). Superworm (*Zophobas morio*) and yellow mealworm (*Tenebrio molitor*) as alternative protein sources. *Animals*, 12(10): 1277. <https://doi.org/10.3390/ani12101277>
- European Commission (2021): Commission Implementing Regulation (EU) 2021/1975 of 12 November 2021 authorising the placing on the market of frozen, dried and powder forms of *Locusta migratoria* as a novel food. *Official Journal of the European Union*, L402.
- European Commission (2022a): Commission Implementing Regulation (EU) 2022/169 of 8 February 2022 authorising the placing on the market of frozen, dried and powder forms of yellow mealworm (*Tenebrio molitor* larva) as a novel food. *Official Journal of the European Union*.
- European Commission (2022b): Commission Implementing Regulation (EU) 2022/188 of 10 February 2022 authorising the placing on the market of frozen, dried and powder forms of *Acheta domesticus* as a novel food. *Official Journal of the European Union*.
- European Commission (2023): Commission Implementing Regulation (EU) 2023/58 of 5 January 2023 authorising the placing on the market of frozen, paste, dried and powder forms of *Alphitobius diaperinus* larvae as a novel food. *Official Journal of the European Union*.
- Fasce, B., Ródenas, L., López, M. C., Moya, V. J., Pascual, J. J., & Cambra-López, M. (2022). Nutritive value of wheat bran diets supplemented with fresh carrots and wet brewers' grains in yellow mealworm. *Journal of Insect Science*, 22(3), 7. <https://doi.org/10.1093/jisesa/ieac022>
- First, T., Van Haeften, A. J., Oonincx, D. G. A. B., Van Loon, J. J. A., Fogliano, V., & Mishyna, M. (2024). Strike while the iron is hot: unraveling the impact of dietary iron on two edible insects. *Journal of Insects as Food and Feed*, 11(7), 1187–1200. <https://doi.org/10.1163/23524588-00001422>
- Gerland, P., Raftery, A. E., Ševčíková, H., Li, N., Gu, D., Alkema, L., Fosdick, B. K. et al. (2014). World population stabilization unlikely this century. *Science*, 346(6206), 234–237. <https://doi.org/10.1126/science.1257469>
- Gomes da Costa Lima, V. M., Gomes de Souza, R., Rodrigues de Oliveira, T., da Costa Feitosa, A., Mayard, H., Andrade dos Santos, R., Bezerra Pinheiro Lima, S. et al. (2024). Zootechnical performance, feed efficiency and larval composition of *Zophobas morio* fed with poultry litter-based diets. *Journal of Insects as Food and Feed*, 10(12), 2157–2166. <https://doi.org/10.1163/23524588-00001070>
- Gourgouta, M., Rumbos, C. I., Oonincx, D. G. A. B., Adamaki-Sotiraki, C., Karapanagiotidis, I. T., & Athanassiou, C. G. (2024). Local agricultural by-products for rearing superworm larvae (*Zophobas morio*): a Greek example. *Journal of Insects as Food and Feed*, 14(1), 1–14. <https://doi.org/10.1163/23524588-00001106>
- Gourgouta, M., Andreadis, S. S., Koutsogeorgiou, E. I., Rumbos, C. I., Grigoriadou, K., Giannenas, I., Bonos, E. et al. (2024). Larval performance of *Zophobas morio* on diets enriched with post-distillation residues and essential oils. *Environmental Science and Pollution Research*, 31(20), 28847–28855. <https://doi.org/10.1007/s11356-024-32603-8>
- Harsányi, E., Juhász, C., Kovács, L., Huzsvai, R., Pintér, G., Fekete, Z.I., Varga, L., Aleksza, C., & Gyuricza, C. (2020). Evaluation of Organic Wastes as Substrates for Rearing *Zophobas morio*, *Tenebrio molitor*, and *Acheta domesticus* Larvae as Alternative Feed Supplements. *Insects*, 11, 604. <https://doi.org/10.3390/insects11090604>
- López-Gámez, G., del Pino-García, R., López-Bascón, M. A., & Verardo, V. (2024). Improving *Tenebrio molitor* growth and nutritional value through vegetable waste supplementation. *Foods*, 13(4), 594. <https://doi.org/10.3390/foods13040594>
- Ministry of Agriculture, Forestry and Water Management, Republic of Serbia (2023). Regulation on the classification and handling of animal by-products and veterinary-sanitary conditions. Official Gazette of the Republic of Serbia, Belgrade.

- Miravalle, E., Balboa, S., Zanetti, M., Otero, A., & Lazzari, M. (2024). New insights on the degradation of polystyrene and polypropylene by larvae of *Zophobas* and gut bacterial consortium. *Journal of Hazardous Materials*, 478, 135475. <https://doi.org/10.1016/j.jhazmat.2024.135475>
- Moruzzo, R., Mancini, S., & Guidi, A. (2021). Edible insects and sustainable development goals. *Insects*, 12, 557. <https://doi.org/10.3390/insects12060557>
- Pascual, J.J., Fasce, B., Martínez-de-Pablo, A., Andrade, C., López, M. C., & Cambra-López, M. (2024). Nutritive value of cereal grains, protein meals and by-products for yellow mealworms. *Journal of Insects as Food and Feed*, 10(6), 949–958. <https://doi.org/10.1163/23524588-00001022>
- Sadat, F., Hosseini, A., Asoodeh, S., Ostad Movahed, M., & Makhdoumi, A. (2024). Plastic polymer degradation by gut bacteria of *Zophobas morio*. *Environmental Science and Pollution Research*, 31(50), 60359–60370. <https://doi.org/10.1007/s11356-024-35244-z>
- Spranghers, T., Moradei, A., Vynckier, K., Boudrez, M., Pinotti, L., & Ottoboni, M. (2024). Amino acid requirements of yellow mealworm larvae. *Journal of Insects as Food and Feed*, 11(6), 1047–1058. <https://doi.org/10.1163/23524588-00001271>
- Veldkamp, T., van Duinkerken, G., van Huis, A., Ottevanger, E., Bosch, G., & van Boekel, T. (2012). Insects as a sustainable feed ingredient in pig and poultry diets: a feasibility study. Wageningen UR Livestock Research, Report 638.

Phenotypic description of the Lipizzan horses from the Republic of Srpska

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Abstract

The Lipizzan horse has a long breeding tradition in the world, but also in the Republic of Srpska. The Lipizzan population in the Republic of Srpska is about 180 horses with full pedigree and DNA parentage tested. The paper aimed to investigate the differences in the morphometric characteristics of Lipizzan horses from the stud farm Vučijak and private breeders. The research was conducted on a total of 56 Lipizzan horses, 28 from the stud farm Vučijak and 28 from private breeding in the Republic of Srpska. Horses with full origin, older than 4 years, were measured. A total of 10 morphometric traits were measured: height of the withers with a stick, height of the withers with tape, height of the back, height of the croup, height of the root of the tail, chest width, width of the shoulders, length of the body, circumference of the chest and cannonbone circumferences. Morphometric traits were carried out with Litdyn's stick and measuring tape. The results showed that there is a statistically highly significant difference in height of the back ($p=0,005$) and circumferences of the chest ($p=0,015$) between stallions from a stud farm and stallions from private breeding. The statistically highly significant difference was recorded in the circumferences of the chest ($p=0.006$) between stud farm and privately breeding mares. This research represents the first phenotypic description of Lipizzan horses from private breeding and these results can be used in further breeding and selection work.

Keywords: Lipizzan horses, morphometric traits, stud farm, private breeding

INTRODUCTION

The Lipizzan horse breed was created in 1580 in Lipica. It was created by crossing Karst mares with Neapolitan, Kladrubian, Spanish, Arabian stallions and among others. By spreading the story about the grace of the Lipizzan horse breed and about its role in dressage, the interest of people from different countries in this breeding increased. The result was the establishment of a large number of state stud farms (Monterotondo, Piber, Fagaras, Karadordevo, Lipik, Đakovo, Vučijak and others). For easier control of genetic material and selection work, Lipizzan horses are divided into sire lines (8 in total) and mare families (over 60). Today, Lipizzan horse breeding is widespread on almost all continents, from state stud farms, through private associations, equestrian clubs, to individuals who breed it for love. The umbrella organization for Lipizzan horse breed is the International Lipizzan Federation (LIF), which gathers all state studs and private breeders who are breeding purebred Lipizzan with confirmed pedigree.

The tradition of breeding Lipizzan horses in the Republic of Srpska dates back to the spring of 1946, when the stud farm Vučijak was founded in Prnjavor. The aim of establishing the stud farm was to improve the horse population at that time as a working horses. With the establishment of the stud farm Vučijak, the breeding of Lipizzan horses began to spread in local region. Horses with full pedigree for the establishment of the stud farm Vučijak were acquired from the stud farms Lipik and Đakovo from Croatia and from the stud farm Lipica from Slovenia. The basic and first herd consisted of 20 mares and

fillies and a few stallions. Today, the stud farm has 110 horses, representatives of 6 sire lines and 15 mare families, with confirmed origins and DNA analysis. Stud farm Vučijak represents the only state stud farm for the Lipizzan horse breed in the Republic of Srpska as well as in Bosnia and Herzegovina. It has great importance for the local and wider community due to the long tradition of breeding and the great potential for the development of tourism. Since 2019, by establishing cooperation with the Faculty of Agriculture in Banja Luka, intensive work has begun on fulfilling the conditions for a full member status of LIF and this goal was reached in October 2023 at the General Assembly held in Bistrita (Romania), (Janković et al., 2025). The Lipizzan breeding tradition of our country, together with seven other countries (Slovenia, Austria, Croatia, Romania, Italy, Hungary, Slovakia), has been on the UNESCO list of intangible cultural heritage since December 2022. In addition to the stud farm Vučijak, the breeding of Lipizzan horses is also carried out by private breeders, who in 2022 founded the Association of Private Breeders of the Lipizzan Horse Breed in the Republic of Srpska. The association has about 60 horses, which are representatives of 6 sire lines and 12 mare families, and they originate from stud farms Vučijak, Lipik, Đakovo, Karadordevo, Kelebija and from private breeders from neighboring countries. In the last few years, private breeders worked on establishing a registry breeding book, they started with DNA parentage testing, and from 2025 they have observer status in LIF.

Exterior, morphometric traits are important elements in the breeding of the Lipizzan horses, which depend on the genotype and breeding conditions. Lipizzans are different in height and body shape depending on the country and their breeding goals. Stud farm Vučijak breeds a smaller-format horse that was intended for work in agriculture. For the improvement of morphometric traits, selection work is important in order to obtain a horse that will serve the purpose. Assessment of morphometric traits whit aim to show the differences between stud farm and private breeding of Lipizzans in different countries (BiH, Croatia, Slovenia, Austria), then uniformity in the closed population of Lipizzan horse was the subject of numerous studies (Čačić 2011, Štrbac et al. 2014, Važić et al. 2016, Rogić et al. 2019, Preradović et al. 2024). Research has also been conducted to show the relationship between head morphometry and skull shape (Liuti et al. 2020), the determination of differences between morphometry and body shape based on photographs (Druml et al. 2015, 2018), assessment of work ability depending on morphometry (Druml et al. 2016), and the relationship between anatomical characteristics and personality (Debeljak et al. 2022). In addition to research related to the Lipizzan horse breed, other scientists have been engaged in morphometric research on other horse breeds, such as: Matsura et al. (2024) compared the values of morphometric measures measured manually in relation to 3D measurement in riding horses; similarly, Ünal et al. (2025); Giontella et al. (2020) compared genetic parameters and inbreeding with morphometric traits in Sardinian Anglo-Arabs; Jensen et al. (2015, 2019) investigated the relationship between morphometric traits and body weight in Iceland horses in Denmark; Guyo et al. (2024) worked on the morphometry of the Ethiopian horse breed; Urbanek et al. (2023) investigated the relationship between morphometric traits and muscular dystrophy in quarter horses; Riberio et al. (2024) described the phenotypic description of the Brazilian Nordestino horse breed from a different perspective; Omidi et al. (2024) compared morphometric measurements and blood count with the degree of obesity in Iranian horse breeds, etc. All this indicates that horses, on the one hand, and morphometric research on the other, are still one of the current methods that modern science deals with. This research aimed to perform a phenotypic description of the existing Lipizzan populations, from stud farm Vučijak and privately bred in the Republic of Srpska, and to describe differences between them. The phenotypic description of the Lipizzan horses from private breeding was done for the first time, and the results of the work will be the first report.

MATERIAL AND METHODS

A total of 56 Lipizzan horses were measured, 28 were from the stud farm Vučijak (14 stallions and 14 mares) and 28 from private breeders (also 14 stallions and 14 mares). All horses were older than 4 years, had a confirmed origin and DNA parentage tested. Total of 10 traits were measured, of which height at withers (HW), height of back (HB), height at croup (HC), tail root height (HTR), width of chest (WC), width of shoulder (WS) and body length (BL) were measured with a measuring stick, while height at withers second (HWt), chest circumference (CC) and cannon bone circumference (CBC) were measured with a measuring tape. The horses are positioned on a flat surface and are measured from the right and

front sides. Results were compared with results for Lipizzan horses from others scientists. Descriptive statistics were performed for each measure, and differences between stud farm and privately bred horses were tested using the t-test, separately for mares and stallions. P-values used in analysis. The data were statistically processed in MS Excel.

RESULTS AND DISCUSSION

In order to see the differences and the variability of the analyzed measures between stallions and mares from the stud farm and private breeding, descriptive statistics were made for 10 analyzed traits (tables 1 and 2). Results showed that the average values for the withers height with a measuring stick and tape, the height of the back, the height of the croup, the height of the root of the tail, body length and cannon bone in stallions from stud farm are slightly higher compared to privately bred stallions. While chest width, shoulder width and chest circumference were higher in private bred stallions. The results of the t-test showed statistically highly significant differences in height of the back ($p=0,005$) and chest circumference ($p=0,015$) between stallions. Results for other traits were higher than $p=0.05$ and they are without statistically significant differences. By comparing the results for all measures based on the coefficient of variation, the population of stallions from stud farm breeding is more uniform, which was expected because it is a closed population. On the other hand, the privately bred population originated from different countries, so the obtained phenotypic variability was expected. Comparing the obtained results with the standards of LIF (2010), it can be noted that the Lipizzan population in the Republic of Srpska is lower in withers height.

Table 1. Morphometric traits of Lipizzan stallions from stud farm Vučijak ($n=14$) and private breeding ($n=14$)

Measurements (cm)	Stud farm Vučijak			Private breeding			P value
	X	SD	CV	X	SD	CV	
HWt	161 ^a	2.45	1.52%	158.93 ^a	5.12	3.22%	0.374
HW	152.5 ^a	2.59	1.70%	149.57 ^a	3.52	2.36%	0.050
HB	143.79 ^a	2.36	1.64%	139.71 ^b	3.24	2.32%	0.005
HC	149.86 ^a	2.35	1.57%	147.00 ^a	4.13	2.81%	0.080
HTR	143.29 ^a	3.43	2.39%	141.00 ^a	4.40	3.12%	0.184
WC	42.93 ^a	2.81	6.55%	43.36 ^a	1.98	4.58%	0.564
WS	43.07 ^a	3.89	9.04%	43.50 ^a	3.16	7.26%	0.680
BL	148.14 ^a	5.30	3.58%	147.93 ^a	5.46	3.69%	0.913
CC	178.36 ^a	5.73	3.21%	183.57 ^b	4.89	2.67%	0.015
CBC	20.61 ^a	0.71	3.45%	20.36 ^a	0.72	3.53%	0.265

n – number of horses; X – average; SD - standard deviation; CV – coefficient of variation; ^a and ^b indicate statistically significant differences: ^{aa} has no statistically significant difference, ^{ab} has a statistically significant difference

The results presented by Važić et al. (2016) show that the average height of the withers (with a stick) in stallions at the Vučijak stud was 148.8 cm, and it is 3.7 cm lower than the results obtained in this research. According to the same authors, the width of the stallion's chest (39.3 cm) is slightly larger in today's stallions, while the values obtained for the length of the body (153.50 cm) and circumference of the chest (176.90 cm) are slightly smaller today. According to Čačić (2011), the withers height, chest width, croup height, circumference of the chest and cannon bone circumference in stallions from the stud farm Lipik were 151.9, 44.7, 149.5, 188.1 and 19.3 cm, and from the stud farm Đakovo were 155.8, 46.4, 154.1, 1912 and 20.6 cm. Comparing them with the obtained results, it can be concluded that the Lipizzan from the Vučijak is more similar in body format to the Lipizzan from the Lipik than from the Đakovo stud farm. Also, according to Čačić (2011), the morphometric measurements of private breeding stallions from Croatia, the withers height (153.6 cm), chest width (44.4 cm) are higher than the obtained results for

stallions from private breeding in the Republic of Srpska. Štrbac et al. (2014) found that the average withers height of Lipizzan horses is 150.92 cm, and it is lower than the average withers height of stud farm stallions, and that it is higher than the average of withers height of privately bred stallions. According to the same authors, the circumference of the chest (178.79 cm) is greater than the same trait in the stallion from stud farm bred, while it is smaller in stallions from private breeding. Also, cannon bone circumference (18.84 cm) is smaller compared to the obtained values for stallions of both breeding type.

Based on the results in table 2, it can be seen that the average values of the withers height with measuring stick, the withers height with a tape and the back height in mares from stud farm Vučijak are higher than the same average values in private breeding mares. While the croup height, the height of the root of the tail, the chest width, the shoulders width, the length of the body, the chest circumference and cannon bone circumference are higher in mares from private breeding. The highest phenotypic variation of a trait within both populations was expressed in the width of the chest of mares from stud farm Vučijak, while the lowest variation was in the height of the withers measured with a stick in the same group. The results of the t-test showed that there is a statistically highly significant difference in chest circumference ($p=0.006$) between stud farm and private breeding. Results for other traits don't have statistically significant differences. Similar to stallions, the population of mares from stud farm breeding is also more uniform (because breeding is based on closed population), which was also expected.

Table 2. Morphometric traits of Lipizzan mares from stud farm Vučijak (n=14) and private breeding (n=14)

Measurements (cm)	Stud farm Vučijak			Private breeding			P value
	X	SD	CV	X	SD	CV	
HWt	159.21 ^a	3.56	2.23%	157.43 ^a	3.41	2.17%	0.171
HW	150.64 ^a	2.44	1.62%	149.57 ^a	4.22	2.82%	0.457
HB	143.14 ^a	4.07	2.85%	140.71 ^a	3.87	2.75%	0.178
HC	148.86 ^a	3.23	2.17%	149.29 ^a	4.32	2.90%	0.765
HTR	141.64 ^a	3.65	2.58%	142.21 ^a	4.68	3.29%	0.730
WC	39.93 ^a	1.77	4.44%	41.14 ^a	3.92	9.53%	0.242
WS	40.29 ^a	4.81	11.94%	42.82 ^a	4.84	11.31%	0.174
BL	145.57 ^a	4.72	3.24%	149.00 ^a	5.55	3.72%	0.124
CC	179.43 ^a	6.16	3.43%	187.43 ^b	8.83	4.71%	0.006
CBC	19.39 ^a	0.49	2.51%	19.89 ^a	0.96	4.85%	0.116

n – number of horses; X – average; SD - standard deviation; CV – coefficient of variation; ; ^a and ^b indicate statistically significant differences: ^{aa} has no statistically significant difference, ^{ab} has a statistically significant difference

According to Važić et al. (2016), the withers height measured with a stick (147.12 cm) in mares at the Vučijak stud farm is 3.52 cm, are slightly lower than today's mares, while the withers height measured with a tape (154.67 cm) is also higher in today's mares. According to the same authors, chest width (36.90 cm) and chest circumference (175.32 cm) are also higher in today's mares. According to Rogić et al. (2019) withers height (144 cm), chest width (38 cm) and chest circumference (174.67 cm) for the Lipa mare family and withers height (150.33 cm), chest width (40.33 cm) and chest circumference (176.33 cm) for the Ukrina mare family were smaller compared to the obtained results, which means that the Lipizzan format at the stud farm is improved in breeding with selection work. According to Čačić (2011), the withers height measured with a stick (147.2 cm) and a tape (158.2 cm), chest circumference (177 cm) and cannon bone circumference (18.7 cm) of mares from the Lipik stud farm are smaller compared to the results obtained for today's mares from stud farm breeding. According to the same author, the withers height measured with a stick (150.5 cm) and the withers height measured with a tape (160.0 cm) in mares from private breeding in Croatia were bigger compared to mares from private breeding in the Republic of Srpska, while chest circumference (185.8 cm) and cannon bone circumference (19.3 cm) were smaller.

CONCLUSION

The results showed that there are statistically highly significant differences in the height of back and chest circumference between stallions from stud farm breeding and stallions from private breeding. A statistically highly significant difference was also found in the chest circumference between mares from stud farm and private breeding. Based on the obtained results, it can be concluded that both populations of horses are similar. The population of horses bred at stud farm is more uniform in morphometric traits due to the closed population, while the population of horses bred privately is heterogeneous due to the different origins of the horses. In relation to the morphometry standards of the classic Lipizzan horse prescribed by the LIF, the Lipizzan population from the Republic of Srpska is smaller in size and because of that, it is authentic. This paper presents for the first time a phenotypic description of Lipizzan horses from private breeding in the Republic of Srpska. The obtained results represent a preliminary report, and in the future is necessary to increase the population of measured stallions and mares in order to achieve greater reliability of the obtained results.

REFERENCES

- Čačić, M. (2011). Genetička analiza lipicanaca u Hrvatskoj. Doktorska disertacija. Sveučilište u Zagrebu. Agronomski fakultet. <https://doi.org/10.13140/RG.2.2.31696.94729>
- Debeljak, N., Košmerlj, A., Altimiras, J., & Župan Šemrov, M. (2022). Relationship between anatomical characteristics and personality traits in Lipizzan horses. *Scientific Reports*, 12:12618. <https://doi.org/10.1038/s41598-022-16627-z>
- Druml, T., Dobretsberger, M., & Brem, G. (2015). The use of novel phenotyping methods for validation of equine conformation scoring results. *Animal*, 9(6), 928–937. <https://doi.org/10.1017/S1751731114003309>
- Druml, T., Dobretsberger, M., & Brem, G. (2016). Ratings of equine conformation – new insights provided by shape analysis using the example of Lipizzan stallions. *Archives Animal Breeding*, 59, 309–317. <https://doi.org/10.5194/aab-59-309-2016>
- Druml, T., Horna, M., Grilz-Seger, G., Dobretsberger, M., & Brem, B. (2018). Association of body shape with amount of Arabian genetic contribution in the Lipizzan horse. *Archives Animal Breeding*, 61, 79–85, 2018. <https://doi.org/10.5194/aab-61-79-2018>
- Giontella, A., Sarti, F. M., Biggio, G. P., Giovannini, S., Cherchi, R., Pieramati, C., & Silvestrelli, M. (2020). Genetic parameters and inbreeding effect of morphological traits in Sardinian Anglo Arab. *Animals*, 10, 791. <http://dx.doi.org/10.3390/ani10050791>
- Guyo, M., Tareke, M., Tonamo, A., Bediye, D., & Defar, G. (2024). Evaluations of Morphometric Traits and Body Conformation Indices of Horse Ecotypes Reared in the Highlands of Bale Eco-Region, Ethiopia. *Veterinary Medicine and Science*, 10, e70114. <https://doi.org/10.1002/vms3.70114>
- Janković, S., Rogić, B., & Važić, B. (2025). Historical development and significance of the Vučijak stud farm. Symposium Lipicanec 2025 Lipica, Slovenija.
- Jensen, R. B., Danielsen, S. H., & Tauson, A. H. (2015). Body condition score, morphometric measurements and estimation of body weight in mature Icelandic horses in Denmark. *Acta Veterinaria Scandinavica*, 58(Suppl 1), 59. <https://doi.org/10.1186/s13028-016-0240-5>
- Jensen, R., Rockhold, L., & Tauson, A. H. (2019). Weight estimation and hormone concentrations related to body condition in Icelandic and Warmblood horses: a field study. *Acta Veterinaria Scandinavica*, 61, 63. <https://doi.org/10.1186/s13028-019-0498-5>
- Liuti, T., & Dixon, M. P. (2020). The use of the geometric morphometric method to illustrate shape difference in the skulls of different-aged horses. *Veterinary Research Communications*, 44, 137–145. <https://doi.org/10.1007/s11259-020-09779-8>
- Matsuura, A., Torii, S., Ojima, Y., & Kiku, Y. (2023). 3D imaging and body measurement of riding horses using four scanners simultaneously. *Journal of Equine Science*, 35(1), 1–7. <https://doi.org/10.1294/jes.35.1>

- Omidi, A., Rasooli, A., Nazifi, S., Heydari, A., & Seirafinia, M. (2024). Relationship between morphometric measurements and blood parameters in horses with varying adiposity levels and physiological conditions. *Veterinary Medicine and Science*, 10, e70024. <https://doi.org/10.1002/vms3.70024>
- Preradović, S., Rogić, B., Važić, B., & Čutković, M. (2024). Influence of Lipizzan stallions on body measurements of foals. XIII International Symposium on Agricultural Sciences AgroReS 2024, Book of Proceedings, 184-192. <https://doi.org/10.7251/ZARS2401184P>
- Ribeiro, N., Rodrigues de Medeiros, G., Vieira da Silva, M., Soares, K., Arandas, J., Vieira do Nascimento, G., & Ribeiro, M. (2024). Phenotypic characterisation and production system of the Nordestino horse from a multivariate perspective. *Scientific Reports*, 14, 1173. <https://doi.org/10.1038/s41598-023-51018-y>
- Rogić, B., Važić, B., & Ravić, I. (2019). Estimation of phenotypic variability of body measurements in Lipizzan mares. *Biotechnology in Animal Husbandry*, 35 (4), 399-408. <https://doi.org/10.2298/BAH1904399R>
- Ünal, N., Paksoy, Y., & Güngör, O. (2025). Agreement of the morphometric dimensions determined by traditional and image analysis methods for Arabian and Thoroughbred horses. *Archives Animal Breeding*, 68, 253–261. <https://doi.org/10.5194/aab-68-253-2025>
- Urbanek, N., & Zebeli, Q. (2023). Morphometric Measurements and Muscle Atrophy Scoring as a Tool to Predict Body Weight and Condition of Horses. *Veterinary Sciences*, 10, 515. <https://doi.org/10.3390/vetsci10080515>
- Važić, B., Sarajlić, Đ., & Rogić, B. (2016). Morphometric characterization of the Lipizzaner horse breed in the stud „Vučijak". *Biotehnology in Animal Husbandry*, 32 (2), 219-227. <https://doi.org/10.2298/BAH1602219V>
- Štrbac, Lj., & Trivunović, S. (2014). Analysis of some morphological traits of Lipizzaner horses in Vojvodina. 7th International Scientific/Professional Conference Agriculture in Nature and Environment Protection, Vukovar, 28th - 30th May 2014, Proceedings, 116– 121.
- Lipizzan International Federation (LIF 2010). <https://www.lipizzan-online.com/main.asp>

Evaluation of the body conformation of Arabian horses originating from Borike Stud Farm based on body indices

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Abstract

The Arabian horse originated from stud farm Borike, or Boric Arabian horse, has a long tradition of breeding in the territory of Bosnia and Herzegovina (BiH), and the first stud farm for breeding these horses was established in Sarajevo at the end of the 19th century. A significant role in the development of this horse population was played by the stud farm Borike, which ceased operations in 2018. Today, the Borik Arabian horses have been preserved in BiH thanks to private breeding programs. This study aimed to examine, using body indices, whether there is morphometric similarity between Borik Arabian horses (RS population) and traditional Arabian horses bred in the Republic of Croatia (CRO population) whose founders originate from the stud farm Borike. In this study, values were calculated for nine body indices: chest index (CI), body index (BI), compactness or eurysoy index (CEI), dactylo-thoracic index or bone index (DTI), caliber index I (CaI-1), caliber index II (CaI-2), cannon bone load index (CBLI), volume index (VI), and format index (FI). Based on the conducted research and statistical significance testing of the observed differences, it can be concluded that for most indices there is a statistically significant or highly significant difference (CI, BI, CEI, CaI-1, CaI-2, CBLI, VI, and FI). The obtained results indicate that there is a significant difference in body measurements between the two analyzed horse populations and may be applied in the process of defining compliance with the criteria for breed recognition prescribed by the legal framework.

Keywords: Body indices, Borik Arabian horses, traditional Arabian horses

INTRODUCTION

The Borik Arabian horses represent a type of Arabian horse that is not registered with the World Arabian Horse Organization (WAHO), and it is characterized by exceptional endurance, adaptability, and good overall health status (Žiga et al., 2008). The first stud farm for Arabian horses in Bosnia and Herzegovina was established at the end of the 19th century in Sarajevo, with an initial capacity of 60 Arabian stallions. Due to a lack of space in Butmir, the stud farm was relocated in 1898 to Borike near Rogatica, marking the first recorded mention of Borike as a horse-breeding site. In 1900, the stud farm was moved to Goražde, and subsequently to the areas of Modriča, Goražde, and Sarajevo, until its final relocation to Borike in 1930 (Telalbašić et al., 2008). The Borike stud farm officially ceased operations in 2018. Today, the survival of the Arabian horse from Borike in the territory of the Republic of Srpska is the result of the dedication and enthusiasm of private breeders, who, through continuous efforts to preserve the genetic and morphological characteristics of this population, contribute to the protection and promotion of this invaluable heritage (Čutković & Rogić, 2024).

An examination of the stud books of the Borike stud farm shows that numerous sire lines and mare families were recorded in the past. However, according to Čutković (2024), only some of them have been preserved to this day, including six sire lines: Saabich, Lenkoran, Gazal, Kuhaylan Zaid, Mabrouk, and Siglavy, as well as four mare families: Hamdani, O'Bajan, Luna, and representatives of the Hamda Essamra En Nedjed family (Kadina and El Hafi). With the establishment of the Association of Breeders of the Borik Arabian horses of the Republic of Srpska in 2021, activities were initiated with the aim of ultimately restoring the breeding of this horse population to proper and sustainable development (Čutković et al., 2025).

Breeding of the Arabian horse in the Republic of Croatia began in the Višnjica stud (near Slatina) in 1970, when two Arabian mares (509 Hamdani XIV and 468 El Hafi XLII) were imported from the stud farm Borika in Bosnia and Herzegovina (Korabi, 2012). According to Korabi (2012), bearing in mind the specific history of breeding, there was a debate in the circles of breeders in Croatia about the appropriate name for these horses. In the existing regulations of the Republic of Croatia, the breed was registered as "Arabian horse", and among breeders it was often given the prefix traditional Arabian horse due to the breeding tradition in the Republic of Croatia, but also Boric Arabian horse due to its origins in the stud farm Borika in Bosnia and Herzegovina (Korabi, 2012).

Morphometric research of this horse population was the subject of numerous studies in the past (Hrasnica 1937; Bartolović 1962; Telalbašić and Pajanović 1981; Rastija et al. 1992; Telalbašić et al. 2008). However, the results of phenotypic characterization of the recent population are rare and sporadic (Čutković et al., 2025). On the other hand, morphometric and body index analyses are very common working methods in domestic animal research, especially in horses. Thus, Sobczuk and Komosa (2012), Siqueira and Cordeiro (2024) investigated morphometric measures in Arabian horses, while Rezende et al. (2016) studied conformational indices in different genetic groups. Also, Prisacaru (2013) analyzed the body indices of Romanian sports horses of the Shagya breed. Guyo et al. (2024) studied body conformation index evaluations of horses bred in Ethiopia. Morphometric measures were also a subject of different studies of Lipizzan horses (Zechner et al., 2001; Vazić et al., 2016; Rogić et al., 2019).

To define the fulfillment of the conditions for the recognition of a new breed prescribed by the legal framework, it is necessary, among other things, to determine the morphometric uniformity of the existing horse population in the last three generations (Rules, Official Gazette of RS, No. 16/2011). Therefore, this work aims to conduct a comparative analysis of body indices of Borik Arabian horses located in the territory of the Republic of Srpska and of traditional Arabian horses originating from the stud farm Borike and bred in the territory of the Republic of Croatia.

MATERIAL AND METHODS

For the research, the values of a total of nine conformation indices were calculated (Korabi, 2012): chest index (CI), body index (BI), compactness or eurysoy index (CEI), dactylo-thoracic index or bone index (DTI), caliber index I (CaI-1), caliber index II (CaI-2), cannon bone load index (CBLI), volume index (VI), and format index (FI), based on previously taken morphometric measurements (withers height, withers height, chest width, length trunk, chest depth, chest girth, chest girth). Body length and height were measured using a Lydtin stick, while girth was measured using a tape measure. The formulas used for calculating the indices are presented in Table 1. The research included two populations of Arabian horses: the population of Boric Arabian horses from the territory of the Republic of Srpska (RS population) and the CRO population, which includes traditional Arabian horses registered in the registry books of Croatia. A total of 155 horses (stallions and mares) were measured, including 58 members of the RS population and 97 members of the CRO population, older than four years and without visible physical deformities. The body mass of the animals was calculated based on the formula shown in Table 1.

To analyze the significance of the differences in the body indices between the two populations of horses, a t-test was used. The interpretation of results was based on the following thresholds of p-values:

- Not significant: Results with $p \geq 0.05$ were considered statistically insignificant, indicating insufficient evidence to reject the null hypothesis.
- Statistically significant: Results with $p < 0.05$ were considered statistically significant, suggesting that the probability of the observed difference occurring by chance is less than 5%.

- Highly significant: Results with $p < 0.01$ were considered highly significant, providing strong evidence against the null hypothesis, with less than 1% probability of random occurrence.

Microsoft Excel was used for data processing.

Table 1. Formula for calculation of body indices (Korabi, 2012)

Body Index	Formula
Chest index (CI)	Chest width $\times$ 100 / chest depth
Body index (BI)	Body length $\times$ 100 / chest circumference
Compactness or eurysoy index (CEI)	Chest circumference $\times$ 100 / body length
Dactylo-thoracic index or bone index (DTI)	Cannon bone circumference $\times$ 100 / chest circumference
Caliber index I (CaI-1)	Body mass (kg) / withers height
Caliber index II (CaI-2)	(Chest circumference / withers height) $\times$ (cannon bone circumference / withers height) $\times$ 1000
Cannon bone load index (CBLI)	Cannon bone circumference $\times$ 100 / body mass (kg)
Volume index (VI)	Body length $\times$ chest depth $\times$ chest width / 1000
Format index (FI)	Body length $\times$ 100 / withers height
Body mass (kg) (BM)	(Chest circumference ² $\times$ body length + 22.9) / 10835.81

RESULTS AND DISCUSSION

The results of testing the obtained differences for nine body indices are shown in Table 2. Based on the results, it can be concluded that for eight (CI, BI, CEI, CaI-1, CaI-2, CBLI, VI and FI) of the nine analyzed indices there is a statistically significant ($p < 0.05$) or highly significant ($p < 0.01$) difference between the analyzed horse populations.

Table 2. Body indices of RS and CRO populations

	Number of samples		Average		Standard deviation		Coefficient of variation		P value
	RS	CRO	RS	CRO	RS	CRO	RS	CRO	
CI	58	88	56.38	58.72	5.63	4.76	9.98%	8.11%	0.010
BI	58	96	80.48	85.96	3.93	3.55	4.89%	4.13%	0.000
CEI	54	99	124.86	113.00	6.22	20.63	4.98%	18.26%	0.000
DTI	58	107	10.26	10.28	0.65	0.54	6.33%	5.25%	0.855
CaI-1	58	96	2.54	2.70	0.28	0.28	11.07%	10.43%	0.001
CaI-2	58	104	143.52	138.77	13.43	9.56	9.35%	6.89%	0.020
CBLI	58	96	4.82	4.46	0.49	0.42	10.21%	9.38%	0.000
VI	58	89	323.01	370.39	49.28	49.28	15.26%	13.31%	0.000
FI	58	100	95.00	99.70	3.23	3.48	3.40%	3.49%	0.000

The significance of the difference was not recorded only for the dactylo-thoracic index (DTI). Furthermore, the Croatian horse (CRO) population had higher values for the CI, BI, CaI-1, VI and FI indices compared to the population of Arabian horses from Borike (RS population). On the other hand, the results showed that RS population has higher values for CEI, CaI-2 and VI indices compared to the Croatian (CRO) population. Significant differences ($p < 0.05$) for the displayed index were recorded for the CEI index. Such deviations can be explained by different approaches when measuring the body length. Given that the CRO population is more developed and in better condition according to the measures shown, this leads to greater

muscle mass at the end points that are taken when measuring the length of the trunk, so this could have led to a significant difference. The higher body index of the CRO population compared to the Borik Arabian horses (RS population) points to a relatively more elongated physique. When it comes to the dactylo-thoracic index (DTI), no significance of the obtained differences was observed, which indicates a similar level of bone mass development in both studied populations.

The higher value of the cannon bone load index (CBLI) in RS population (4.82 compared to 4.46) indicates a higher relative load of the distal part of the limb, that is, a more robust bone structure in relation to the body mass. The volume index (VI) in the CRO population is 370.39, while in the RS population it is 323.01, which indicates more pronounced proportions and overall physical development in the CRO population. Higher values of the format index (FI) in traditional Arabian horses (CRO population) point to a more harmonious ratio of body height and length, i.e. to a more pronounced rectangular body format.

Comparing the obtained results with other research, it can be concluded that similar values for BI (85), CaI-1 (300), and DTI (11) were also reported by Chaabi et al. (2025) for Algerian Fantasia horses. The values of the CEI index for Romanian sport horses (112.10) and Romanian Shagya horses (119.96) are similar to those of Croatian traditional Arabian horses (CRO population), while they are lower compared to Borik Arabian horses (RS population). The values of the FI index for Romanian sport horses (99.60) are similar to those of Croatian traditional horses (CRO population), whereas the values for Shagya horses (114.52) are significantly higher (Prisacaru, 2013). According to the classification of horses based on DTI presented in Rezenda et al. (2016), it can be concluded that both investigated horse populations belong to the group of eumetric animals, i.e., animals of medium, proportionate body type, in which the skeleton and body mass are in harmonious proportion.

CONCLUSION

Based on the conducted analysis and obtained results for nine conformation indices, it can be concluded that there is a statistically significant or highly significant difference in most indices (CI, BI, CEI, CaI-1, CaI-2, CBLI, VI and FI). No statistically significant difference was found in dactylo-thoracic index (DTI), which may indicate the similarity of the studied populations in this parameter. During the comprehensive analysis, it was observed that the population of traditional Arabian horses has (CRO population) a higher average value in CI, BI, DTI, CaI-1, VI, FI, while in CEI, CaI-2 and CBLI the population of Borik Arabian horse (RS population) has a higher average value. The obtained results indicate that there is a clear morphometric differentiation between the analyzed populations, which may be a consequence of different growing conditions, nutrition, and breeding goals. These results contribute to a better understanding of the variability of the studied populations and represent a significant basis for further scientific research.

REFERENCES

- Bartolović, T. (1962). O razvitku arapske ždrebadi u prvoj godini života. *Veterinaria*, 1, 8-10.
- Chaabi, A. Z., Boukhechem, S., Bougherara, H., Afoutni, L., Djeghar, A.E., Boukerrou, M., Tekkouk Zemmouchi, F., & Dib, A. L. (2025). Morphometric characterization of Algerian fantasia horses at a national equestrian competition. *Biology and Life Sciences Forum*, 49(1), 7. <https://doi.org/10.3390/blsf2025049007>
- Ćutković, M. (2024). Genealogija boričkog arapskog konja (Graduation thesis). Poljoprivredni fakultet, Univerzitet u Banjoj Luci.
- Ćutković, M., & Rogić, B. (2024). Genealoška stabla linija pastuva boričkog arapskog konja. Students in Encounter with Science – StES 2024, Proceedings, 27–36. University of Banja Luka.
- Ćutković, M., Rogić, B., Važić, B., & Janković, S. (2025). Morphometric description of the Borik Arabian horse mare families. XIV International Symposium on Agricultural Sciences – AgroReS 2025, Proceedings, 193 - 197. <https://doi.org/10.63356/agrores.2025.026>

- De Siqueira, R. F., & Cordeiro, N. D. M. S. (2024). Linear measurements, body proportions, and biomechanical insights: morphological analysis of Arabian horses in São Paulo, Brazil. *Brazilian Journal of Veterinary Research and Animal Science*, 61, e223460. <https://doi.org/10.11606/issn.1678-4456.bjvras.2024.223460>
- Guyo, M., Tareke, M., Tomano, A., Bediye, D., & Defar, G. (2024). Evaluations of morphometric traits and body conformation indices of horse ecotypes reared in the highlands of Bale Eco-Region, Ethiopia. *Veterinary Medicine and Science*, 10(6). <https://doi.org/10.1002/vms3.70114>
- Hrasnica, F. (1937). Prilog poznavanju eksterijera, mehaničkih odnosa i duljine koraka kod našeg arapskog konja. *Veterinarski Arhiv*, 9-10, 197-237.
- Korabi, N. (2012). Genetska i morfološka analiza tradicionalnih arapskih, punokrvnih arapskih i shagya-arap konja u Republici Hrvatskoj (Doctoral dissertation). Agronomski fakultet, Sveučilište u Zagrebu.
- Prisacaru, M. C. (2013). The analysis of corporal indices of stallions from Rădăuți troop. *Animal Science Series*, 59, 190-192.
- Rastija, T., Baban, M., & Barišić, A. (1992). Reproduktivne osobine i tjelesne mjere arapskih konja. In: Borički arapski konj - Zbornik radova, 301-303.
- Rezende, M. P. G., Souza, J. C., Mota, M. F., Oliveira, N. M., & Jardim, R. J. D. (2016). Conformation index of horses of different genetic groups. *Ciência Animal Brasileira*, 17(3), 316-326. <https://doi.org/10.1590/1089-6891v17i321194>
- Rogić, B., Važić, B., & Ravić, I. (2019). Estimation of phenotypic variability of body measurements in Lipizzan mares. *Biotechnology in Animal Husbandry*, 35(4), 399-408. <https://doi.org/10.2298/BAH1904399R>
- Rules on the conditions for the recognition of new breeds, lines and hybrids of domestic animals. Official Gazette of RS, No. 16/2011)
- Sobczuk, D., & Komosa, M. (2012). Morphological differentiation of polish Arabian horses – multivariate analysis. *Bulletin of the Veterinary Institute in Pulawy*, 56(4), 623-629. <https://doi.org/10.2478/v10213-012-0110-5>
- Telalbašić, R., & Pajanović, R. (1981). Prilog poznavanju stanja uzgoja i tipa arapske ergele na Borikama za vrijeme trajanja posljednjeg generacijskog intervala. 32. Zasjedanje Evropske stočarske federacije.
- Telalbašić, R., Žiga, E., & Rahmanović, A. (2008). Historijat ergele “Borike” i uzgoj Arapskog konja od 1895. do 2007. godine. *Stočarstvo*, 62(5), 417-429.
- Važić, B., Sarajlić, Đ., & Rogić, B. (2016). Morphometric characterization of the Lipizzaner horse breed in the stud “Vučijak”. *Biotechnology in Animal Husbandry*, 32(2), 219-227. <https://doi.org/10.2298/BAH1602219V>
- Zechner, P., Zohman, F., Sölkner, J., Bodo, I., Habe, F., Martifs, E., & Brem, G. (2001). Morphological description of the Lipizzan horse population. *Livestock Production Science*, 69, 163-177. [https://doi.org/10.1016/S0301-6226\(00\)00254-2](https://doi.org/10.1016/S0301-6226(00)00254-2)
- Žiga, E., Telalbašić, R., & Rahmanović, A. (2008). Borički arapski konj. Sarajevo/Zagreb.

Social network analysis in pigs: Insights into social organisation, behaviour, and welfare in pig production systems

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Abstract

Social network analysis (SNA) has become an increasingly valuable approach for studying social interactions in animal ethology. By moving beyond simple dyadic relationships, SNA enables the analysis of entire social systems and allows researchers to quantify both direct and indirect interactions among individuals. This approach provides insights into individual social roles, subgroup formation, and the stability of group dynamics. In pigs, social interactions during lactation, weaning, and the post-weaning period play a crucial role in shaping welfare, growth, and productivity. Applying SNA to pig ethology therefore offers new opportunities for understanding how social structures emerge and how management practices influence group stability. This review summarises current knowledge on the application of social network analysis in pigs, focusing on early social interactions, suckling ethology regrouping at weaning, and post-weaning social dynamics. The potential of SNA for improving welfare assessment and supporting management strategies in pig production systems is also discussed.

Keywords: social network analysis, piglets, early socialisation, weaning, pig ethology

INTRODUCTION

Social network analysis (SNA) provides a powerful framework for quantifying relationships among individuals and understanding the structure of social systems. Rooted in graph theory (Freeman, 1984; Newman, 2010), SNA allows researchers to move beyond simple dyadic interactions and capture indirect social connections, such as relationships among individuals that share common partners (Brent et al., 2015). By applying standardized mathematical measures across different levels of social organisation, from individuals to entire populations, SNA enables detailed and comparative analyses of social ethology (Makagon et al., 2012; Croft et al., 2016).

Originally developed within sociology and psychology (Wasserman & Faust, 1994; Scott, 2000), social network approaches have since been applied across a wide range of scientific disciplines, including epidemiology, information sciences, economics, and biology (Barabási & Oltvai, 2004; Ings et al., 2009; Martínez-López et al., 2009). Within animal ethology research, SNA has gained increasing attention over the past two decades, particularly in studies of socially complex species such as primates and cetaceans (Flack et al., 2005; Lusseau, 2003).

More recently, social network approaches have also been applied in livestock research, providing new insights into the social ethology of farm animals. In pigs, SNA can be used to analyse patterns of association, aggression, and affiliative interactions, as well as the effects of management practices such as early socialisation, cross-fostering, and regrouping after weaning. Because social interactions strongly influence welfare, health, and productivity in pig production systems, understanding these interactions is of considerable practical importance.

The aim of this review is to summarise current knowledge on the application of social network analysis in pigs. Particular emphasis is placed on the use of SNA to study piglet behaviour during lactation,

weaning, and the post-weaning period, as well as on its potential for improving welfare assessment and management strategies in modern pig production systems.

Development of social network research in pigs

Social network analysis (SNA), originally developed within sociology and psychology (Wasserman & Faust, 1994; Scott, 2000), has been widely applied across various scientific disciplines, including epidemiology, information sciences, economics, and biology (Barabási & Oltvai, 2004; Ings et al., 2009; Martínez-López et al., 2009). Over the past two decades, SNA has also gained increasing attention in animal ethology and ecology, particularly in studies of socially complex species such as primates and cetaceans (Flack et al., 2005; McCowan et al., 2011; Lusseau, 2003). By focusing on relationships among individuals rather than isolated behavioural events, SNA enables researchers to capture indirect connections within groups and to analyse how individual interactions shape broader social organisation.

In livestock research, however, the application of SNA has developed more slowly. Early studies in pig ethology primarily relied on dyadic observations or pen-level assessments of aggression and harmful behaviours such as tail or ear biting (Brunberg et al., 2011; Zonderland et al., 2011; Munsterhjelm et al., 2016). These behaviours often occur unpredictably and can spread rapidly within groups, making them difficult to analyse using traditional behavioural approaches (D'Eath et al., 2014). Consequently, network-based approaches provide an important opportunity to examine social interactions at the group level and to better understand the mechanisms underlying these behaviours.

Initial applications of SNA in pigs focused mainly on agonistic interactions and basic network properties within groups (Büttner et al., 2015a; Büttner et al., 2015b). These studies demonstrated that aggressive behaviour is not randomly distributed but organised within structured social networks, in which certain individuals occupy central or intermediary positions. Identifying such individuals can provide valuable insights into dominance relationships and the stability of group hierarchies.

Building on this approach, Agha et al. (2020) showed that an individual's position within aggression networks is closely associated with economically important production traits. Their results indicated that pigs occupying less conflict-intensive positions within social networks exhibited better growth performance and fewer injuries. This finding highlighted the practical relevance of SNA by linking behavioural network dynamics directly to production efficiency.

Subsequent research expanded beyond aggression-centred analyses to include affiliative interactions and physiological mechanisms underlying social behaviour. Camerlink et al. (2014) demonstrated that social conflict in pigs involves not only aggressive behaviour but also affiliative nosing interactions that may help reduce social tension. This work suggested that social organisation following regrouping reflects a dual-process system in which affiliative ties contribute to group stabilisation alongside dominance interactions.

Further evidence has emphasised the biological foundations of social relationships in pigs. Camerlink et al. (2016) demonstrated that intranasal oxytocin administration increases affiliative contact and reduces aggression, suggesting that positive social ties may be hormonally regulated. Similarly, Veit et al. (2021) found that immune activation can temporarily alter pigs' positions within social networks, reducing centrality and overall social engagement. These findings indicate that physiological states can influence social network structure and should therefore be considered when interpreting network metrics in livestock populations.

Recent studies have also explored the predictive potential of social network analysis. Foister et al. (2018) showed that aggression observed within the first 24 hours after mixing can predict levels of aggression several weeks later. These results highlight the potential of network-based indicators for identifying socially influential individuals and predicting long-term behavioural outcomes within pig groups.

The developmental basis of social relationships has also attracted increasing attention. Camerlink et al. (2023) reported that affiliative preferences established before weaning predict calmer behavioural and physiological responses during later separation events. This suggests that early-life social networks may contribute to social resilience and facilitate smoother social transitions during regrouping.

Early research in pig ethology has also highlighted the importance of environmental conditions and early social development for shaping behaviour and social interactions, including the effects of enrichment and early-life experiences (Peterson et al., 1989; Fraser et al., 1991).

Beyond growing pigs, social network approaches have also been applied to commercial sow populations. Jowett et al. (2023) observed that sows maintained stable affiliative partnerships despite repeated regrouping in dynamic housing systems, indicating that social preferences in pigs can persist even under fluctuating social conditions.

Methodological developments have further refined the application of SNA in pig research. Recent studies increasingly distinguish between spatial proximity and intentional physical contact. While proximity networks often reflect shared use of space or resources, contact networks provide a more accurate representation of selective social affiliations. For example, Khatiwada et al. (2025) showed that temporal patterns of snout contact shortly after mixing can predict later network centrality, suggesting that social organisation begins to stabilise within hours of regrouping. Similarly, Camerlink et al. (2025) found that contact-based networks reveal meaningful affiliative structures, particularly after weaning when familiarity and reciprocity influence group formation more strongly than simple co-location.

Recent advances in automated monitoring and analytical approaches have further expanded the application of SNA in pig research, with AI-assisted methods revealing hidden social structures and detailed behavioural patterns (Agha et al., 2025; Jowett et al., 2025).

Together, these studies demonstrate that pig social networks are structured, dynamic, and biologically meaningful. The integration of behavioural, physiological, developmental, and methodological perspectives has substantially improved our understanding of pig social systems and highlights the potential of SNA as a valuable tool for welfare assessment and management in modern pig production systems.

Social network analysis (SNA)

Social network analysis (SNA) is a methodological framework used to quantify relationships among individuals within a group (Freeman, 1984; Wasserman & Faust, 1994; Borgatti et al., 2013; Newman, 2010). By representing individuals as nodes and their interactions as edges, SNA enables researchers to examine the structure and dynamics of social systems beyond simple dyadic relationships. This approach allows the identification of an individual's position within the network and provides insights into how interaction patterns influence group organisation, social stability, and behavioural outcomes (Croft et al., 2016; Makagon et al., 2012).

In animal ethology, SNA offers important advantages over traditional behavioural observations because it captures both direct and indirect social connections. This makes it possible to identify central individuals, socially peripheral animals, and potential bridges linking different subgroups within a population (Brent et al., 2015; Croft et al., 2008). Consequently, SNA has become an increasingly valuable tool for analysing complex social interactions in group-living species, including pigs.

Basic characteristics of social networks

Social networks consist of nodes (individuals) connected by edges representing social interactions (Wasserman & Faust, 1994; Borgatti et al., 2013). The structure of these networks is shaped by several properties describing how individuals interact with one another.

One important property is assortativity or homophily, where individuals preferentially associate with others that share similar characteristics such as age, sex, or body mass (Newman, 2010). Another key feature is reciprocity, which describes the mutual exchange of interactions between two individuals (Croft et al., 2008). The strength of a social tie is typically determined by the frequency or intensity of interactions and may be expressed as a weighted relationship within the network (Granovetter, 1973).

Networks may also contain both strong and weak ties. Strong ties generally represent frequent interactions among familiar individuals, whereas weak ties often connect otherwise separate parts of the network and can facilitate the spread of information or behaviours between subgroups (Granovetter, 1973).

Closedness

Closedness refers to the tendency for individuals who share common partners to also interact with each other, forming closed triads within the network (Wasserman & Faust, 1994). Such clustering of relationships often indicates stronger social cohesion and stability within a group.

Bridges

Bridges represent individuals or connections that link otherwise separate parts of a network. These connections play an important role in maintaining overall network connectivity and allow information or behaviours to spread between different subgroups (Borgatti et al., 2013; Granovetter, 1973).

Centrality

Centrality measures describe the relative importance or influence of an individual within a social network (Freeman, 1984). Different centrality metrics capture different aspects of an individual's role in the network structure. Centrality and network parameters are used to describe the position of individuals and the overall structure of social networks. The most commonly applied metrics in studies of pigs include degree, betweenness, and closeness centrality, as well as measures of clustering, cohesion, and subgroup structure (Wasserman & Faust, 1994; Borgatti et al., 2013; Croft et al., 2016).

Table 1. Commonly used SNA parameters in pig studies

Parameter	Description
All-degree centrality	The number of edges which are attached to a node
In-degree centrality	The number of head ends adjacent to a node, which represent the number of received agonistic action of the current pig.
Out-degree centrality	The number of tail-ends adjacent to a node, which represent the number of initiated agonistic behaviours with other pen-mates.
Eigenvector centrality	The connectivity of an individual within its network, according to the all-degree centrality of the animal and the all-degree centrality of the animals that connect with.
Betweenness centrality	The number of shortest paths that pass through the considered animal. This measures the importance of the animal in connecting different subgroups the pen in aggression.
Closeness centrality	Proximity to all individuals in the network
Clustering coefficient	Degree to which social partners are interconnected (triads)
Network cohesion	Overall level of connectedness within the group
Community detection	Identification of subgroups within the network
Clique size	Size of fully connected subgroups

Compiled based on standard SNA methodology (Freeman, 1984; Wasserman and Faust, 1994, Borgatti et al., 2013, Newman, 2010) and its application in pig ethology studies (Büttner et al., 2015a; Büttner et al., 2015b; Foister et al., 2018; Agha et al., 2020).

Application of social network analysis in piglets

The behaviour of piglets during lactation, weaning, and the post-weaning period provides an important framework for applying social network analysis (SNA). Piglets live in dynamic social groups where interactions are frequent and often associated with competition for resources such as teats or feed. SNA enables researchers to quantify these interactions and analyse both individual roles within the group (e.g., centrality or dominance position) and broader group-level structures such as network density, clustering, and subgroup formation (Makagon et al., 2012; Croft et al., 2008).

Lactation and suckling behaviour

Newborn piglets establish a suckling order shortly after birth (Skok and Škorjanc, 2014). This order typically stabilises within the first week of lactation and reduces competition among littermates while improving survival (De Passille et al., 1988; Puppe and Tuchscherer, 1999; Skok and Gerken, 2016). From a social network perspective, suckling behaviour can be interpreted as a structured system of repeated interactions centered on access to teats as contested resources.

Piglets that consistently occupy preferred anterior teats may represent more central individuals within the interaction network, whereas piglets occupying posterior teats may assume more peripheral positions, which can influence their growth and survival (Fraser et al., 1991; Peterson et al., 1989). In addition, repeated nursing in proximity to the same littermates may lead to the formation of small and stable interaction clusters, reflecting early social organisation within the litter.

Management practices such as cross-fostering may also influence these early interaction networks. Cross-fostering is commonly used to equalise litter size and reduce variation in piglet body weight (Neal and Irvin, 1991; Robert and Martineau, 2001; Souza et al., 2014). When performed shortly after birth, before the establishment of a stable suckling order, it can improve survival among lighter piglets. However, if performed later, it may disrupt established social relationships and teat fidelity, potentially increasing competition and instability within the developing social network.

Weaning

Weaning represents a major transition in pig production, during which piglets are separated from the sow and mixed with unfamiliar individuals. This process disrupts previously established social relationships and requires the formation of new dominance hierarchies, often resulting in aggressive interactions (Colson et al., 2006; Olsen et al., 1998; Telkänranta and Edwards, 2018).

Social network analysis provides a useful framework for examining these interactions at the group level. Rather than analysing aggression as isolated dyadic events, SNA allows researchers to examine entire aggression networks and identify individuals occupying key structural positions. Individuals with high degree centrality may participate in many aggressive interactions, whereas individuals with high betweenness centrality may act as bridges connecting different parts of the network.

Network-level measures such as density, clustering coefficient, and centralisation can reveal whether newly formed groups become cohesive or remain socially fragmented during this transitional phase.

Early socialisation

Early socialisation, where piglets are exposed to unfamiliar littermates before weaning, represents one of the most effective welfare-friendly strategies for reducing aggression after regrouping. Piglets with early social experience are better able to establish new social relationships and often rely more on affiliative interactions such as play rather than aggression when encountering unfamiliar individuals (Weary et al., 2002; D'Eath, 2005; Peterson et al., 1989).

From a network perspective, early socialisation influences the structure of interaction networks formed after mixing. Socialised piglets typically form more connected and cohesive networks, reflected in higher network density and clustering. At the same time, lower network centralisation indicates that aggressive interactions are distributed more evenly across individuals rather than concentrated in a few dominant animals (Verdon et al., 2016; van Nieuwamerongen et al., 2015).

Temporal network analyses further suggest that individuals establishing affiliative ties early may contribute to long-term group stability. Contact networks indicate that partially familiar regrouping can reduce stress after mixing by preserving existing social bonds (Khawwaja et al., 2025). Socialised piglets also show fewer injuries, faster stabilisation of dominance hierarchies, and improved growth performance compared with non-socialised animals (Morgan et al., 2014; Salazar et al., 2018).

Post-weaning period

During the post-weaning period, aggressive interactions play a central role in establishing social hierarchies within pig groups. Although aggression is a natural component of social organisation, excessive aggression can lead to injuries, increased stress responses, and reduced growth performance (Marchant et al., 1995; Mendi et al., 1992; Tan et al., 1991; Stookey et al., 1994).

Social network analysis allows these interactions to be studied at the group level by identifying individuals that are highly involved in aggressive exchanges or that connect otherwise separate subgroups within the network. Changes in network structure may also influence physiological and production-related outcomes, including immune responses and carcass quality (Morrow-Tesch et al., 1994; D'Eath et al., 2014).

Overall, applying SNA to piglet behaviour across these developmental stages provides valuable insights into the formation and stabilisation of social structures. By analysing both individual roles and group-level patterns, network approaches can improve the understanding of social dynamics in pig production systems and contribute to the development of welfare-oriented management strategies.

CONCLUSION

Social network analysis (SNA) provides a powerful framework for understanding the complex social organisation of pigs. By quantifying relationships among individuals, SNA enables researchers to identify key social roles, subgroup structures, and interaction patterns that shape group stability. These insights are particularly valuable in pig production systems, where social interactions strongly influence welfare, health, and productivity.

Applications of SNA in pig research have demonstrated that social networks are structured and dynamic, with individual positions within the network influencing behavioural outcomes such as aggression, social stability, and group cohesion. Studies have also shown that management practices—including early socialisation, regrouping strategies, and environmental conditions—can significantly affect network structure and the distribution of social roles within groups.

Recent advances in monitoring technologies, including RFID systems, computer vision, and automated behavioural tracking, create new opportunities for applying social network analysis in predictive welfare assessment.

Overall, the growing body of research indicates that SNA represents a valuable tool for both scientific investigation and practical management of pig social systems. Continued development of network-based approaches, particularly through longitudinal studies and automated monitoring technologies, will further enhance our ability to understand, predict, and manage social dynamics in pig populations.

REFERENCES

- Agha, S., Fàbrega, E., Quintanilla, R., & Sánchez, J. P. (2020). Social network analysis of agonistic behaviour and its association with economically important traits in pigs. *Animals*, 10(11), 2123. <https://doi.org/10.3390/ani10112123>
- Agha, S., Psota, E., Turner, S. P., Lewis, C. R., Steibel, J. P., & Doeschl-Wilson, A. (2025). Revealing the hidden social structure of pigs with AI-assisted automated monitoring data and social network analysis. *Animals*, 15(7), 996. <https://doi.org/10.3390/ani15070996>
- Barabási & Oltvai (2004) Barabási, A.-L., & Oltvai, Z. N. (2004). Network biology: Understanding the cell's functional organization. *Nature Reviews Genetics*, 5(2), 101–113. <https://doi.org/10.1038/nrg1272>
- Borgatti, S. P., Everett, M. G., & Johnson, J. C. (2013). Analyzing social networks. Sage Publications.
- Brent, L. J. N. (2015). Friends of friends: Are indirect connections in social networks important to animal behaviour? *Animal Behaviour*, 103, 211–222 <https://doi.org/10.1016/j.anbehav.2015.01.020>

- Brunberg, E., Wallenbeck, A., & Keeling, L. J. (2011). Tail biting in fattening pigs: Associations between frequency of tail biting and other abnormal behaviours. *Applied Animal Behaviour Science*, 133(1–2), 18–25. <https://doi.org/10.1016/j.applanim.2011.04.019>
- Büttner, K., Scheffler, K., Czycholl, I., & Krieter, J. (2015a). Social network analysis – centrality parameters and individual network positions of agonistic behaviour in pigs over three different age levels. *SpringerPlus*, 4, 185. <https://doi.org/10.1186/s40064-015-0979-4>
- Büttner, K., Czycholl, I., Mees, K., & Krieter, J. (2015b). Temporal development of social networks in pigs after weaning using social network analysis. *Applied Animal Behaviour Science*, 171, 83–91. <https://doi.org/10.1016/j.applanim.2015.08.006>
- Camerlink, I., Reimert, I., & Bolhuis, J. E. (2016). Intranasal oxytocin administration in relation to social behaviour in domestic pigs. *Physiology & Behavior*, 163, 51–55. <https://doi.org/10.1016/j.physbeh.2016.04.040>
- Camerlink, I., Scheck, K., Cadman, T., & Rault, J.-L. (2023). Effects of preferential social associations on pigs' response to weaning. *Animal*, 17(6), 100846. <https://doi.org/10.1016/j.animal.2023.100846>
- Camerlink, I., Turner, S. P., Ursinus, W. W., Reimert, I., & Bolhuis, J. E. (2014). Aggression and affiliation during social conflict in pigs. *PLOS ONE*, 9(11), e113502. <https://doi.org/10.1371/journal.pone.0113502>
- Camerlink, I., Turner, S. P., Lee, V., & Khatiwada, S. (2025). Contact-based social networks reveal affiliative structures in pigs after weaning. *Animal* (in press).
- Colson, V., Orgeur, P., Courboulay, V., Dantec, S., Foury, A., & Mormède, P. (2006). Grouping piglets by sex at weaning reduces aggressive behaviour. *Applied Animal Behaviour Science*, 97, 152–171. <https://doi.org/10.1016/j.applanim.2005.08.006>
- Croft, D. P., James, R., & Krause, J. (2008). Exploring animal social networks. Princeton University Press.
- Croft, D. P., Darden, S. K., & Wey, T. W. (2016). Current directions in animal social networks. *Current Opinion in Behavioral Sciences*, 12, 52–58. <https://doi.org/10.1016/j.cobeha.2016.09.001>
- De Passillé, A. M. B., Rushen, J., & Hartsock, T. G. (1988). Ontogeny of teat order in pigs and its relation to competition at suckling. *Applied Animal Behaviour Science*, 19, 325–338. [https://doi.org/10.1016/0168-1591\(88\)90059-3](https://doi.org/10.1016/0168-1591(88)90059-3)
- D'Eath, R. B. (2005). Socialising piglets before weaning improves social hierarchy formation after mixing. *Applied Animal Behaviour Science*, 93(3–4), 199–211. <https://doi.org/10.1016/j.applanim.2004.11.019>
- D'Eath, R. B., Arnott, G., Turner, S. P., Jensen, T., Lahrmann, H. P., Busch, M. E., Niemi, J. K., Lawrence, A. B., & Sandoe, P. (2014). Injurious tail biting in pigs: How can it be controlled in existing systems without tail docking? *Animal*, 8, 1479–1497. <https://doi.org/10.1017/S1751731114001352>
- Freeman, L. C. (1984). Centrality in social networks: Conceptual clarification. *Social Networks*, 1, 215–239. [https://doi.org/10.1016/0378-8733\(78\)90021-7](https://doi.org/10.1016/0378-8733(78)90021-7)
- Flack, J. C., Girvan, M., de Waal, F. B. M., & Krakauer, D. C. (2005). Policing stabilizes construction of social niches in primate societies. *Nature*, 439, 426–429. <https://doi.org/10.1038/nature04326>
- Fraser, D., Phillips, P. A., Thompson, B. K., & Tennessen, T. (1991). Effect of straw on the behaviour of growing pigs. *Applied Animal Behaviour Science*, 30(3–4), 307–318. [https://doi.org/10.1016/0168-1591\(91\)90106-6](https://doi.org/10.1016/0168-1591(91)90106-6)
- Foister, S., et al. (2018). Social network properties predict chronic aggression in commercial pig systems. *PLOS ONE*, 13(10), e0205122. <https://doi.org/10.1371/journal.pone.0205122>
- Granovetter, M. S. (1973). The strength of weak ties. *American Journal of Sociology*, 78(6), 1360–1380. <https://doi.org/10.1086/225469>
- Ings, T. C., Montoya, J. M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C. F., Edwards, F., Figueroa, D., Jacob, U., Jones, J. I., Lauridsen, R. B., Ledger, M. E., Lewis, H. M., Olesen, J. M., Van Veen, F. J. F., Warren, P. H., & Woodward, G. (2009). Ecological networks – beyond food webs. *Journal of Animal Ecology*, 78, 253–269. <https://doi.org/10.1111/j.1365-2656.2008.01460.x>

- Jowett, S. L., Barker, Z. E., & Amory, J. R. (2023). Preferential associations in a dynamic sow herd. *Frontiers in Veterinary Science*, 10, 1166632. <https://doi.org/10.3389/fvets.2023.1166632>
- Jowett, S. L., Silk, M. J., Lee, V., Turner, S. P., & Camerlink, I. (2025). Characterising nosing behaviours in pigs after mixing using social network analysis. *Animal*, 19(8), 101585. <https://doi.org/10.1016/j.animal.2025.101585>
- Khatiwada, S., Lee, V., Turner, S. P., & Camerlink, I. (2025). Temporal social network structures based on snout contact after mixing. *Applied Animal Behaviour Science*, 106838. <https://doi.org/10.1016/j.applanim.2025.106838>
- Lusseau, D. (2003). The emergent properties of a dolphin social network. *Proceedings of the Royal Society B: Biological Sciences*, 270, 186–188. <https://doi.org/10.1098/rsbl.2003.0057>
- Makagon, M. M., McCowan, B., & Mench, J. A. (2012). How can social network analysis contribute to social behaviour research in applied ethology? *Applied Animal Behaviour Science*, 138, 152–161. <https://doi.org/10.1016/j.applanim.2012.02.003>
- Martínez-López, B., Perez, A. M., & Sánchez-Vizcaíno, J. M. (2009). Social network analysis: Review of general concepts and use in preventive veterinary medicine. *Transboundary and Emerging Diseases*, 56, 109–120. <https://doi.org/10.1111/j.1865-1682.2009.01073.x>
- Marchant, J. N., Rudd, A. R., Mendl, M. T., Broom, D. M., Meredith, M. J., Corning, S., & Simmins, P. H. (1995). Timing and causes of piglet mortality in alternative farrowing systems. *Veterinary Record*, 136, 209–212. <https://doi.org/10.1136/vr.136.9.209>
- Mendl, M., Zanella, A. J., & Broom, D. M. (1992). Physiological and reproductive correlates of behavioural strategies in female domestic pigs. *Animal Behaviour*, 44, 1107–1121. [https://doi.org/10.1016/S0003-3472\(05\)80323-7](https://doi.org/10.1016/S0003-3472(05)80323-7)
- Morgan, T., Camerlink, I., Turner, S. P., Bolhuis, J. E., & Lawrence, A. B. (2014). Socialising piglets during lactation: Effects on behaviour after mixing and on welfare. *Applied Animal Behaviour Science*, 158, 23–33. <https://doi.org/10.1016/j.applanim.2014.05.004>
- Morrow-Tesch, J., McGlone, J. J., & Norman, R. L. (1994). Consequences of social stress on behaviour and immunity in pigs. *Journal of Animal Science*, 72, 1513–1519. <https://doi.org/10.2527/1994.7261513x>
- Munsterhjelm, C., Heinonen, M., & Valros, A. (2016). Application of the Welfare Quality® protocol to pigs: Tail biting and other welfare indicators. *Applied Animal Behaviour Science*, 174, 79–87. <https://doi.org/10.1016/j.applanim.2015.10.012>
- Neal, M. E., & Irvin, K. M. (1991). The effects of cross-fostering on piglet survival and growth. *Journal of Animal Science*, 69, 41–47. <https://doi.org/10.2527/1991.69141x>
- Newman, M. (2010). *Networks: An Introduction*. Oxford University Press, Oxford.
- Olsen, A. N., Dybkjær, L., & Vestergaard, K. S. (1998). Cross-suckling behaviour. *Applied Animal Behaviour Science*, 61, 13–24. [https://doi.org/10.1016/S0168-1591\(98\)00169-7](https://doi.org/10.1016/S0168-1591(98)00169-7)
- Salazar, L. C., et al. (2018). Early socialisation increases social skills. *Applied Animal Behaviour Science*, 206, 25–31. <https://doi.org/10.1016/j.applanim.2018.05.002>
- Scott, J. (2000). *Social network analysis: A handbook* (2nd ed.). Sage Publications.
- Scott, K., Taylor, L., Gill, B. P., & Edwards, S. A. (2009). Environmental enrichment in finishing pigs. *Applied Animal Behaviour Science*, 116, 186–190. <https://doi.org/10.1016/j.applanim.2008.08.002>
- Skok, J., & Škorjanc, D. (2013). Teat order modelling in piglets. *Applied Animal Behaviour Science*, 144, 39–45. <https://doi.org/10.1016/j.applanim.2012.12.007>
- Skok, J., & Škorjanc, D. (2014). Modelling the development of teat order and its influence on piglet growth. *Applied Animal Behaviour Science*, 154, 36–44. <https://doi.org/10.1016/j.applanim.2014.02.007>
- Skok, J., & Gerken, M. (2016). Relationship between teat order stability and piglet survival and growth. *Applied Animal Behaviour Science*, 175, 36–42. <https://doi.org/10.1016/j.applanim.2015.12.012>

- Stookey, J. M., Gonyou, H. W., & Jones, S. D. M. (1994). The effects of regrouping pigs on behaviour, growth, and meat quality. *Applied Animal Behaviour Science*, 40, 13–24. [https://doi.org/10.1016/0168-1591\(94\)90066-3](https://doi.org/10.1016/0168-1591(94)90066-3)
- Souza, L. P., et al. (2014). Cross-fostering strategies and their effects on piglet survival and performance. *Livestock Science*, 167, 117–122. <https://doi.org/10.1016/j.livsci.2014.06.011>
- Peterson, V., Fraser, D., & Pajor, E. A. (1989). The development of social behaviour in piglets and its relation to play and aggression. *Applied Animal Behaviour Science*, 23, 67–75. [https://doi.org/10.1016/0168-1591\(89\)90070-1](https://doi.org/10.1016/0168-1591(89)90070-1)
- Puppe, B., & Tuchscherer, M. (1999). Development of social hierarchy in suckling piglets. *Applied Animal Behaviour Science*, 63, 265–278. [https://doi.org/10.1016/S0168-1591\(99\)00012-9](https://doi.org/10.1016/S0168-1591(99)00012-9)
- Robert, S., & Martineau, G. P. (2001). Effects of cross-fostering on piglet survival and growth performance. *Canadian Journal of Animal Science*, 81, 71–73. <https://doi.org/10.4141/A00-011>
- Tan, S. S. L., Shackleton, D. M., & Beames, R. M. (1991). The effects of mixing unfamiliar pigs on growth and behaviour. *Applied Animal Behaviour Science*, 30, 307–318. [https://doi.org/10.1016/0168-1591\(91\)90126-K](https://doi.org/10.1016/0168-1591(91)90126-K)
- Telkänranta, H., & Edwards, S. A. (2018). Weaning of pigs: Behavioural and welfare implications. *Applied Animal Behaviour Science*, 207, 1–9. <https://doi.org/10.1016/j.applanim.2018.06.013>
- Van Nieuwamerongen, S. E., Bolhuis, J. E., Van der Peet-Schwering, C. M. C., & Soede, N. M. (2015). Effects of early social contact on piglet behaviour and social competence after weaning. *Applied Animal Behaviour Science*, 166, 44–51. <https://doi.org/10.1016/j.applanim.2015.03.007>
- Van de Weerd, H. A., & Day, J. E. L. (2009). A review of environmental enrichment for pigs housed in intensive housing systems. *Applied Animal Behaviour Science*, 116, 1–20. <https://doi.org/10.1016/j.applanim.2008.08.001>
- Veenema, A. H. (2009). Early life stress, the development of aggression and neuroendocrine mechanisms. *Frontiers in Neuroendocrinology*, 30(4), 497–518. <https://doi.org/10.1016/j.yfrne.2009.05.002>
- Veit, C., et al. (2021). Immune activation alters social network structure in pigs. *Animal*, 15(9), 100332. <https://doi.org/10.1016/j.animal.2021.100332>
- Verdon, M., Morrison, R. S., & Hemsworth, P. H. (2016). Rethinking group housing of pigs: The role of early socialisation. *Applied Animal Behaviour Science*, 174, 1–10. <https://doi.org/10.1016/j.applanim.2015.10.010>
- Wasserman, S., & Faust, K. (1994). *Social Network Analysis: Methods and Applications*. Cambridge University Press.
- Weary, D. M., Pajor, E. A., & Thompson, B. K. (1999). Behaviour of piglets in communal areas. *Applied Animal Behaviour Science*, 65, 123–135. [https://doi.org/10.1016/S0168-1591\(99\)00063-4](https://doi.org/10.1016/S0168-1591(99)00063-4)
- Zonderland, J. J., Schepers, F., Bracke, M. B. M., Den Hartog, L. A., Kemp, B., & Spoolder, H. A. M. (2011). Characteristics of tail biting behaviour in pigs and the effects on welfare. *Applied Animal Behaviour Science*, 133, 54–61. <https://doi.org/10.1016/j.applanim.2011.04.005>

The impact of learning theory knowledge on improved horse welfare

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Abstract

In recent years, increased attention has been directed toward horse welfare in equestrian sport by both researchers and practitioners. However, welfare remains difficult to define and assess, as it encompasses physiological, behavioural, and cognitive components. Within this review, key findings from equine learning theory are presented, with emphasis on their relevance for everyday interactions with horses and their potential contribution to improved welfare. Although substantial progress has been made in understanding equine learning processes through controlled studies, the application of this knowledge in practical settings remains limited. One of the central issues is the regulation of the relationship between motivation and stress. While a certain degree of stress may facilitate learning, excessive or poorly managed stress has been associated with reduced performance and compromised well-being. For this reason, training approaches should be designed in a way that considers the horse's behavioural tendencies and physiological constraints. In equestrian sport, multiple stakeholders are involved; nevertheless, performance-oriented goals are still frequently prioritised over welfare considerations. This situation underlines the importance of approaches that are both effective and scientifically informed. Principles derived from learning theory have been shown to provide practical guidance for improving training methods, handling practices, and overall management. Greater integration of these concepts into educational and professional frameworks is considered necessary in order to reduce the gap between scientific knowledge and its application in daily practice. When applied consistently, learning theory principles can support clearer communication, more efficient learning, and improved long-term welfare outcomes. In conclusion, equine learning theory represents a scientifically grounded framework for advancing welfare in equestrian sport, while also supporting more responsible and sustainable practices.

Keywords: horse welfare, human-animal interaction, learning theory

INTRODUCTION

Horse welfare remains a central topic of ongoing scientific investigation and debate, yet fundamental questions persist regarding how it can be reliably assessed and effectively improved. Perspectives on equine welfare have evolved considerably over the past fifty years, reflecting advances in research, changing societal expectations, and shifts in industry practices. Horses occupy a distinctive status in human society, eliciting strong emotional responses, and although scientists and legislators increasingly emphasize evidence-based practices, traditional habits and popular beliefs remain influential (Lesimple, 2020). Poor horse welfare, along with compromised safety for riders and other participants, continues to challenge the social acceptance of the equine industry (Hall-Bromley and Dixon, 2025; Luke et al., 2023; Broom, 2011; Gordon, 2001). Evidence suggests that these issues are interconnected, with improvements in horse welfare likely to enhance human safety (Luke et al., 2023; Henshall et al., 2022). The Five Domains Model, encompassing nutrition, physical environment, health, behavioral interactions, and mental state, provides a comprehensive framework for evaluating welfare (Mellor et

al., 2020). However, recent studies indicate that equestrian participants' conceptualizations of welfare frequently diverge from this model; their stated priorities often do not align with practical implementation, and discrepancies exist between public statements and private beliefs regarding horse welfare (Luke et al., 2023). Unlike companion animals such as dogs and cats, horses evolved as prey species, resulting in a unique and complex dynamic in human-horse interactions that raises important theoretical and applied scientific questions (Brubaker and Udell, 2016). Training, riding, and breeding practices constitute critical determinants of equine welfare. Sporadic welfare violations, such as the widely condemned incident during the modern pentathlon at the 2020 Tokyo Olympic Games, highlight ongoing challenges to the social legitimacy of equestrian sport (Foreman, 2024; O'Connell et al., 2025). Defining welfare remains inherently complex. Broadly, it refers to the state of an animal's body and mind at a given moment. Recognizing horses as sentient beings capable of positive and negative experiences is central to promoting well-being. These experiences serve adaptive functions, eliciting physiological and behavioral responses that help maintain or restore homeostasis across cells, tissues, organs, and body systems. Advances in neuroscience now allow for integration of behavioral observations with biological measures to assess whether experiences are aversive or acceptable. Despite this knowledge, Luke et al. (2023) and Girgis et al. (2025) report that learning theory terminology is rarely applied by equestrians, and understanding of learning principles does not consistently translate into improved welfare or safety outcomes. In response, the Fédération Equestre Internationale has established an Equine Ethics and Wellbeing Commission to safeguard the sport's sustainability.

The aim of this paper was to present insights from equine learning theory and their practical application for improving human-horse interactions and enhancing horse welfare, reflecting both current scientific understanding and evidence-based strategies.

EQUINE LEARNING THEORY

Equine learning theory explains how horses process, retain, and react to information through associative (classical/operant conditioning) and non-associative (habituation/sensitization) learning (McLean and Christensen, 2017). Equines are constantly learning from every interaction they have with us, even when we aren't trying to train them. Learning theory is a framework that describes how horses absorb, process and retain information when they are learning. These processes can be influenced by cognitive, emotional and environmental factors, as well as the horse's past experiences with learning and training. The core principles of learning theory apply to all creatures single-celled organisms have demonstrated basic learning capabilities (Copelin, 2025, Dussutour, 2021.) Learning theory supplies a relevant and useful toolbox for trainers and riders, it is crucial to always be mindful that training is essentially an exploitative event and there is no ubiquitous training modality; all have advantages and pitfalls and outcomes may be influenced by arousal, affective and attachment states (McLean and Christensen, 2017, Christensen et al., 2006). According to Doherty et al. (2017) training methods that do not align with the horse's natural learning abilities reduce the likelihood of optimal performance and increase the frequency of problem behaviors as well as jeopardizing equine welfare. To understand equine learning theory, one needs to understand learning abilities in horses, which is now referred to as Gagné's Conditions of Learning Theory (Gagné, 1970, Copelin, 2025), but horses have eight "levels" of learning abilities ranked from least to most complex (Table 1). Equine brains can Habituation (Learning not to respond to a repeated stimulus), classical conditioning (responding to a new stimulus that is paired with a familiar stimulus), operant conditioning (performing a specific action to achieve a desired result), chaining responses (performing a sequence of responses for reinforcement/reward), concurrent discrimination (Identifying differences between two or more similar objects) and concept learning (identifying and categorizing stimuli or objects based on shared characteristics).

Table 1. Learning abilities in horses (McGreevy, 2018; Sappington and Goldman, 1994; Copelin, 2025)

Level of complexity	Description	Example
Habituation	Learning not to respond to a repeated	A horse stops reacting to the loud sound past after hearing it several times
Classical conditioning	Responding to a new stimulus that is paired with a familiar stimulus	The horses get excited about the ritual event (the feedlot door opens)
Operant conditioning	Performing a certain behavior to achieve a desired outcome or avoid an undesired outcome	A treat is awarded for a horse's good performance.
Chaining responses	Performing a sequence of responses for reinforcement-reward	A series of desirable horse actions for a treat
Concurrent discrimination	Identifying and categorizing stimuli or objects based on common characteristics	Grouping otherwise unrelated objects by color
Concept learning	Identifying and categorizing stimuli or objects based on common characteristics	Identifying and categorizing stimuli or objects based on common characteristics
Conditional concepts	Understanding that one situation depends on another, even if the situations are not closely linked in space or time (If XYZ... then ABC)	Understanding related events if we don't prepare food we will go hungry.
Bi-conditional concepts	Logical reasoning and understanding that the reverse of a situation can also be true	Understanding reasoning such as shapes, sides of a sequence, etc.

Equine brain is not capable of higher reasoning and there is no scientific evidence that horses can understand conditional or bi-conditional concepts (levels Conditional concepts and Bi-conditional concepts) (McGreevy, 2018 Sappington end Goldman.1994, Hanggi, 1999. Gregić et al. 2024b., Copelin, 2025). In practice itself according to Girgis, et al., (2025) the results of this study have demonstrated that UK equestrians have a poor understanding of learning theory. Luke et al. (2023) found a poor understanding of learning theory among the Australian general equestrian population. Watney et al. (2025). a deficit knowledge in horse owners in understanding learning theory terminology, pain recognition and what constitutes normal clinical parameters for a “healthy” horse. Poor understanding of learning theory among the equestrian population leads to poor implementation and undermines ethics and horse welfare.

HORSE WELFARE

Negative associations with horse welfare are often linked to stress; however, well-being is a broader concept that is not solely defined by the absence of stress and is frequently compromised by ignorance. The Fédération Equestre Internationale Sports Horse Forum, through the Equine Ethics and Wellbeing Commission, continues to evolve as empirical evidence from welfare science expands (EEWB, 2023). The concept of “A Good Life for Horses” encompasses both the physical and mental state of the horse, as well as the extent to which their natural instincts and inherent needs, or telos, are fulfilled. Telos refers to the unique characteristics of equine anatomy, physiology, ethology, and cognition (Mellor et al., 2020). According to Lesimple (2020), animal welfare can be defined as a chronic state reflecting an animal’s subjective perception of its environment, as indicated by behavioral, postural, and physiological parameters. Horses are continuously exposed to a range of environmental conditions, some of which may inadequately meet their needs and thus compromise welfare. The welfare of ridden horses has been examined from multiple perspectives, including riders’ understanding of training methods, horse management practices, and the use of riding equipment (Luke et al., 2023).

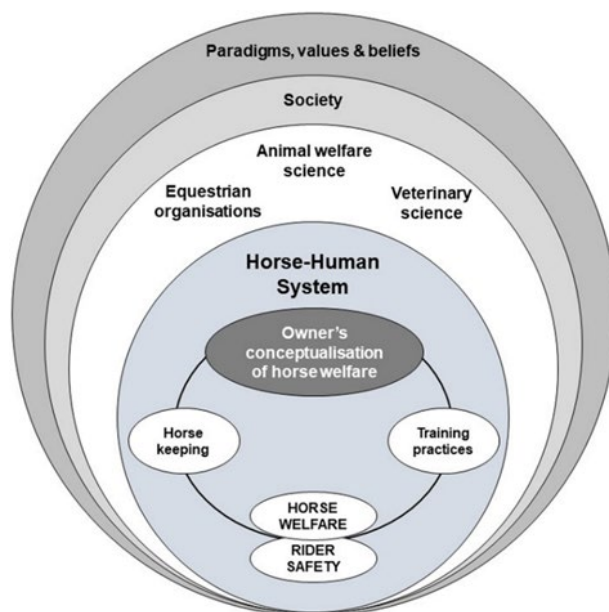


Figure 1. Schematic individual horse-human systems within larger systems, equestrian organisations and society (Luke et al., 2023)

Figure 1 provides a schematic representation of unrecognized relationships and potential points of influence for facilitating positive changes in equestrian sports. Most research, including that of Luke et al. (2023), examines these issues from the perspective of the individual equestrian; however, Figure 1 illustrates that individual equestrians and horses operate within broader systems, including equestrian organizations and society, which are in turn shaped by often unarticulated assumptions and beliefs. Contemporary evaluations of horse welfare reflect perspectives from multiple societal levels, educational contexts, social status, and governmental oversight. Results from Torell Palmquist et al. (2025) highlight the need for a universal cultural shift towards an evidence-based approach to riding and training, with equine welfare as a central consideration. Factors such as anthropomorphism, variability in standards across disciplines and countries, and the evolving nature of the horse-human relationship may compromise the social license to operate within equestrian sports. Societal attitudes are increasingly influenced by digital media and social networks, where content is generated by individuals and can shape public perception of equestrian practices (Gregić et al., 2024a). Torell Palmquist et al. (2025) emphasize the importance of communicating positive outcomes and evidence-based practices to enhance ethical and welfare standards in training and competition. Ethics is often associated with welfare; however, it more broadly refers to the philosophical study of moral concepts, including theories of good and bad, right and wrong, and codified systems of values and principles (Hrvatska enciklopedija, 2026). Equine welfare is both an ethical obligation to the horse and a practical necessity for sustainable equestrian practice. The goal of equine welfare should be to ensure that horses experience a “Good Life,” characterized by predominantly positive experiences over time (EEWB, 2023). Douglas et al. (2022) note that equestrian sport depends on effectively addressing ethical considerations alongside welfare concerns. Luke et al. (2023) further argue that equine welfare is best promoted by addressing issues both within and outside the equestrian community. Research consistently demonstrates the fundamental needs of horses in equestrian sport (Torell Palmquist et al., 2025; Douglas et al., 2022; Luke et al., 2023; Mellor et al., 2020; Hall-Bromley and Dixon, 2025). Watney et al. (2025) highlight the urgent need for practical knowledge on implementing evidence-based approaches to overcome barriers to adoption and improve current practices.

HORSE AND HUMAN INTERACTIONS

Horses have a long and complex history in their interactions with humans. Latella and Abrams (2025) note that just as horse and rider are interlinked through close bodily contact, concepts such as trust, attachment, and nonverbal communication are also evident during unmounted equine interactions. Across different cultures, horses have historically been utilized for labor, cultural rituals, consumption,

sport, therapy, and companionship, and they continue to serve in these roles today (Brubaker and Udell, 2016). Given that horses evolved as prey animals, the horse-human relationship presents unique scientific questions of both theoretical and applied significance. Horses and humans frequently form close bonds, with research demonstrating, for example, that a horse's heart rate can increase in response to a rider's elevated heart rate (Merkies et al., 2014). Understanding this human-horse bond is critical for ensuring the safety and welfare of both species (Hawson et al., 2010; Thompson et al., 2015; Brubaker and Udell, 2016; Mellor et al., 2020; Gregić et al., 2024b). Horses appear to integrate multisensory information, including visual, olfactory, and auditory cues, to distinguish between humans and to assess the attentional state of individuals (Brubaker and Udell, 2016). Experimental evidence indicates that horses are sensitive to prior human interactions associated with positive outcomes, such as food, suggesting their potential for research on individual recognition, theory of mind, and attentional sensitivity, capabilities that are highly relevant for human-horse work. The ability of humans to communicate effectively with horses through gestures and body posture is critical for safe and efficient interactions (Ladewig, 2019). Human-horse interactions, particularly activities such as standing, stroking, or rubbing, may facilitate social bonding in horses without eliciting stress responses in either species (Jung and Yoon, 2025, Hausberger et al., 2008). Awareness of both equine and human body language enhances safety and efficiency in handling horses (Ladewig, 2019, Jardat et al., 2023). Furthermore, environments in which foals and young horses are properly socialized promote the development of social competence, preparing them for adult life within a social group, a prerequisite for optimal horse welfare and effective human-horse interaction.

CONCLUSION

In conclusion, although laboratory knowledge and insights into equine learning theory have expanded considerably, their practical application in horse management remains limited. The distinction between negative stress and motivation for learning is complex, underscoring the need for a proactive approach to mitigate compromised equine welfare resulting from insufficient application of scientific knowledge. Current welfare practices often focus primarily on the horse itself, without adequate attention to work methods or the fulfilment of natural needs, despite the involvement of multiple stakeholders. The rapid advancement of learning theory over the past decade offers substantial potential to improve equine welfare globally. Integrating these scientific insights into educational curricula for equestrians, trainers, and other professionals is essential to translate theory into practice. The urgent development of innovative, evidence-based strategies represents a critical opportunity to enhance horse welfare, promote positive learning experiences, and strengthen human-horse interactions.

ACKNOWLEDGEMENT

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REFERENCES

- Broom, D. M. (2011). A history of animal welfare science. *Acta Biotheoretica*, 59(2), 121-137.
<https://doi.org/10.1007/s10441-011-9123-3>
- Brubaker, L., & Udell, M. A. (2016). Cognition and learning in horses (*Equus caballus*): What we know and why we should ask more. *Behavioural Processes*, 126, 121-131.
<https://doi.org/10.1016/j.beproc.2016.03.017>
- Copelin C. (2025). How Do Horses Learn? The Science Behind Equine Training & Behavior
<https://madbarn.com>. (6.3.2026.)
- Christensen, J. W., Rundgren, M., & Olsson, K. (2006). Training methods for horses: habituation to a frightening stimulus. *Equine Veterinary Journal*, 38(5), 439-443.
<https://doi.org/10.2746/042516406778400574>

- Douglas, J., Owers, R., & Campbell, M. L. (2022). Social licence to operate: what can equestrian sports learn from other industries?. *Animals*, 12, 15, 1987. <https://doi.org/10.3390/ani12151987>
- Doherty, O., McGreevy, P. D., & Pearson, G. (2017). The importance of learning theory and equitation science to the veterinarian. *Applied Animal Behaviour Science*, 190, 111-122. <https://doi.org/10.1016/j.applanim.2017.02.012>
- Dussutour, A., (2021). Learning in Single Cell Organisms. *Biochemical and Biophysical Research Communications*, 564, 92-102. <https://doi.org/10.1016/j.bbrc.2021.02.018>
- EEWB. (2023). Equine Ethics and Wellbeing Commission - Interim Report to the FEI Sports Forum April 2023 by Professor Nat Waran. 2023. Available online: <https://equinewellbeing.fei.org/assets/documents/Interim%20Report%20to%20FEI%20Sports%20Forum%202023%20SLIDES%20including%20notes.pdf> (19.3.2026).
- Foreman, J. H. (2024). Analysis of Tokyo 2020 Olympic modern pentathlon equestrian jumping results. *Journal of Equine Veterinary Science*, 136, 105068. <https://doi.org/10.1016/j.jevs.2024.105068>
- Gabor, V., & Gerken, M. (2010). Horses Use Procedural Learning Rather than Conceptual Learning to Solve Matching to Sample. *Applied Animal Behaviour Science*, 126, 3-4, 119-124. <https://doi.org/10.1016/j.applanim.2010.06.006>
- Gagne, R. M. (1970). The conditions of learning (2nd ed.). Holt, Rinehart & Winston.
- Girgis, E., Shaw, D. J., Heffernan, D., Spence, H., & Pearson, G. (2025). Investigation of a potential link between UK equestrians' understanding of learning theory, and their perception of and response to a problematic equine behaviour. *Applied Animal Behaviour Science*, 287, 106639. <https://doi.org/10.1016/j.applanim.2025.106639>
- Gregić, M., Bobić, T., Gantner, R., & Gantner, V. (2024a). The use of digital media in equestrian clubs in Croatia. *Proceedings*, 94, 34. <https://doi.org/10.3390/proceedings2024094034>
- Gregić, M., Zaharov, T., Gantner, V., & Bobić, T. (2024b). Knowledge and application of horse learning theory. 34th International Scientific-Expert Conference of Agriculture and Food Industry (Agriconference), Book of Abstracts, 108-108
- Hanggi, E. B. (1999). Categorization learning in horses (*Equus caballus*). *Journal of Comparative Psychology*, 113(3), 243–252. <https://doi.org/10.1037/0735-7036.113.3.243>
- Hall-Bromley, A., & Dixon, L. (2025). Education and Licensing of Horse Owners: Addressing Poor Horse Welfare in the UK. *Animals*, 15(7), 1037. <https://doi.org/10.3390/ani15071037>
- Hausberger, M., Roche, H., Henry, S., & Visser, E. K. (2008). A review of the human–horse relationship. *Applied Animal Behaviour Science*, 109(1), 1-24. <https://doi.org/10.1016/j.applanim.2007.04.015>
- Henshall, C., Randle, H., Francis, N., & Freire, R. (2022). Habit formation and the effect of repeated stress exposures on cognitive flexibility learning in horses. *Animals*, 12(20), 2818. <https://doi.org/10.3390/ani12202818>
- Hrvatska enciklopedija, mrežno izdanje. Leksikografski zavod Miroslav Krleža, 2013. – 2026. Pristupljeno. <<https://www.enciklopedija.hr/clanak/etika>>. (19.3.2026)
- Jardat, P., Destrez, A., Damon, F., Menard-Peroy, Z., Parias, C., Barrière, P., & Lansade, L. (2023). Horses discriminate human body odors between fear and joy contexts in a habituation-discrimination protocol. *Scientific Reports*, 13(1), 3285. <https://doi.org/10.1038/s41598-023-30119-8>
- Jung, Y., & Yoon, M. (2025). The Effects of Human–Horse Interactions on Oxytocin and Cortisol Levels in Humans and Horses. *Animals*, 15(7), 905. <https://doi.org/10.3390/ani15070905>
- Ladewig, J. (2019). Body language: Its importance for communication with horses. *Journal of Veterinary Behavior*, 29, 108-110. <https://doi.org/10.1016/j.jveb.2018.06.042>
- Lesimple, C. (2020). Indicators of horse welfare: State-of-the-art. *Animals*, 10(2), 294. <https://doi.org/10.3390/ani10020294>
- Latella, D., & Abrams, B. (2025). The role of the equine in human–animal interactions. In Handbook on animal-assisted therapy, 203-222. Academic Press. <https://doi.org/10.1016/B978-0-443-22346-4.15002-4>

- Luke, K. L., McAdie, T., Warren-Smith, A. K., Rawluk, A., & Smith, B. P. (2023). Does a Working Knowledge of Learning Theory Relate to Improved Horse Welfare and Rider Safety? *Anthrozoös*, 36(4), 703–719. <https://doi.org/10.1080/08927936.2023.2166713>
- O'Connell, E., Dyson, S., McLean, A., & McGreevy, P. (2025). No more evasion: Redefining conflict behaviour in human–horse interactions. *Animals*, 15(3), 399. <https://doi.org/10.3390/ani15030399>
- Mellor, D. J., Beausoleil, N. J., Littlewood, K. E., McLean, A. N., McGreevy, P. D., Jones, B., & Wilkins, C. (2020). The 2020 five domains model: Including human–animal interactions in assessments of animal welfare. *Animals*, 10(10), 1870. <https://doi.org/10.3390/ani10101870>
- Merkies, K., Sievers, A., Zakrajsek, E., MacGregor, H., Bergeron, R., & von Borstel, U. K. (2014). Preliminary results suggest an influence of psychological and physiological stress in humans on horse heart rate and behavior. *Journal of Veterinary Behavior*, 9(5), 242–247. <https://doi.org/10.1016/j.jveb.2014.06.003>
- McLean, A. N., & Christensen, J. W. (2017). The application of learning theory in horse training. *Applied Animal Behaviour Science*, 190, 18–27. <https://doi.org/10.1016/j.applanim.2017.02.020>
- Sappington, B. F., & Goldman, L., (1994). Discrimination Learning and Concept Formation in the Arabian Horse. *Journal of Animal Science*, 72(12), 3080–3087. <https://doi.org/10.2527/1994.72123080x>
- Thompson, K., McGreevy, P., & McManus, P. (2015). A critical review of horse-related risk: A research agenda for safer mounts, riders and equestrian cultures. *Animals*, 5(3), 561–575. <https://doi.org/10.3390/ani5030372>
- Torell Palmquist, G., Käsälä Alveheim, N., Huot-Marchand, F., Ashton, L., & Lewis, V. (2025). The role of European equestrian institutions in training professionals: Outcomes from a workshop on horse welfare in equestrian education. *Animals*, 15(2), 183. <https://doi.org/10.3390/ani15020183>
- Watney, M., Lancaster, B., & Randle, H. (2025). Horse owners' knowledge of fundamental care and their perceptions on the implementation of a mandatory certificate of knowledge. *Journal of Applied Animal Welfare Science*, 28(4), 653–672. <https://doi.org/10.1080/10888705.2024.2393124>

Production performance of broiler chickens under commercial farming conditions in the Banja Luka region

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Abstract

This study aimed to evaluate production performance on three commercial broiler farms of similar capacity (18.000 broilers per production cycle) in the Banja Luka region (Republic of Srpska, Bosnia and Herzegovina) during the period 2022–2025. A total of 60 production cycles were analyzed, comprising five cycles per farm per year. Production parameters included fattening period, final body weight, feed conversion ratio (FCR), first-week mortality, total mortality, and the European Production Efficiency Factor (EPEF). Data were analyzed using descriptive statistics, analysis of variance (ANOVA), and Pearson correlation analysis. The average fattening period was 40.2 days, while the average feed conversion ratio was 1.69, and both parameters were significantly affected only by farm ($P < 0.05$). The average final body weight was 2.30 kg and was influenced only by production year ($P < 0.05$), suggesting the presence of annual variability related to environmental or organizational factors. First-week mortality and total mortality averaged 1.71% and 3.92%, respectively, with only total mortality showing a significant farm effect ($P < 0.05$). The European Production Efficiency Factor averaged 324.79 and was significantly influenced by both farm and production year ($P < 0.05$). Correlation analysis revealed a strong positive relationship between fattening period and final body weight ($r = 0.75$), a positive correlation between fattening period and FCR ($r = 0.37$), and significant correlations of EPEF with final body weight ($r = 0.43$) and FCR ($r = -0.75$) ($P < 0.05$). The results demonstrate that broiler production efficiency is influenced by farm-specific management and annual variation. These findings provide practical insights for optimizing broiler production and emphasize the importance of targeted management strategies to enhance production efficiency.

Keywords: broiler chickens, production performance, feed conversion, mortality, EPEF

INTRODUCTION

Broiler meat is among the three most widely consumed types of meat worldwide, a trend likely driven by its affordability, versatility in culinary preparation, and consumers' perception of broiler meat as a healthy food (Baéza et al., 2022). Commercial broiler meat production is predominantly based on broiler genotypes specifically developed for fattening under intensive production systems, characterized by rapid growth rates and high feed efficiency. As a result, broiler meat remains affordable for consumers across different socioeconomic groups (Choi et al., 2023). In addition, broiler meat production is associated with a lower environmental impact compared to pork and beef production (Neeteson et al., 2023).

The broiler meat available on the market of Bosnia and Herzegovina mainly originates from broiler production system based on cooperation between production organizers and farmers (Zenunović & Glavić, 2017). Within this model, the responsibilities between organizer and farmer are defined by contract, a practice widely adopted in poultry production (Salihbašić et al., 2015; Karaman et al., 2023). During the period from 2011 to 2020, the average annual production of broiler meat in Bosnia and Herzegovina amounted to 54,624 t, with an average annual growth rate of 4.15%, while the degree of self-sufficiency and per capita consumption averaged 90% and 17 kg, respectively (Jalić et al., 2023). Nevertheless, this level of consumption remained lower than that reported in the European Union, where per capita broiler

meat consumption reached 23.5 kg in 2020 (AVEC, 2025). Production results achieved on broiler farms in Bosnia and Herzegovina have generally been at an acceptable level; however, there remains potential for further improvement when the genetic potential of modern broiler hybrids is taken into account (Vekić et al., 2019; 2020). Improved coordination between farmers and the meat processing industry could further stimulate the growth of domestic broiler meat sales and increase the market share of local companies under the current economic and market conditions in the country (Salihbašić et al., 2015).

Production efficiency on broiler farms is commonly assessed using several performance parameters. Feed conversion ratio (FCR) is considered one of the most economically important indicators, as feed costs may account for up to 70% of total variable costs in broiler meat production (Adaszyńska-Skwirzyńska et al., 2025). Moreover, FCR is increasingly used as an indicator of the environmental impact of broiler production, since improvements in feed efficiency are typically associated with reduced negative effects on air, water, and soil quality in the vicinity of broiler farms (Gržinić et al., 2023). Final body weight, fattening period, and broiler mortality are also widely used parameters for evaluating production efficiency on broiler farms (Karaman et al., 2023; Chibanda et al., 2024). The fattening period is generally determined by the target final body weight, which largely depends on consumer preferences and the requirements of the meat processing industry (Van Horne, 2018). First-week mortality and total mortality are considered rough indicators of flock quality and the adequacy of ambient and management conditions during chick placement and the fattening period, making them important factors influencing the economic efficiency of broiler production (Junghans et al., 2022). The European Production Efficiency Factor (EPEF) is a composite index that incorporates the aforementioned parameters in its calculation and is therefore commonly used to assess the technical and economic efficiency of broiler farms (Dotas et al., 2021).

The usefulness of farm-level production data has been well documented in studies aimed at typifying broiler farms according to their technical and economic characteristics (Dotas et al., 2021; Bara et al., 2025), as well as in investigations exploring the relationships between production performance and rearing conditions (Averós & Estevez, 2018; Baracho, 2019; Szöllösi et al., 2021), weather conditions (Campe et al., 2013; Özel & Bozkurt, 2023), and antibiotic use (Roskam et al., 2020). Collectively, these studies have demonstrated that the production efficiency of broiler farms results from the combined influence of multiple elements constituting the production system and that performance can be improved through the identification and mitigation of negative factors (Averós & Estevez, 2018).

The aim of the present study was to determine key production parameters in broiler fattening achieved during the period from 2022 to 2025 on three commercial farms of identical capacity and equipment located in the Banja Luka region (Republic of Srpska, Bosnia and Herzegovina), and to subsequently compare them according to farm and production year in order to assess the current status of broiler production in the studied region.

MATERIALS AND METHODS

The study analyzed three commercial broiler chicken farms located in the Banja Luka region (Republic of Srpska, Bosnia and Herzegovina). The average capacity of the farms was approximately 18,000 broiler chicks per production cycle, reared in a floor housing system. With regard to construction characteristics, the farms were comparable in terms of the building materials, as well as the total housing area available for broiler placement. In terms of technical equipment, heating, ventilation, feeding, and drinking systems were similar across farms, differing only with respect to equipment manufacturers. Straw was used as litter material on all farms, while stocking density ranged from 14 to 18 birds per square meter. A lighting program consisting of 22 hours of continuous light and 2 hours of darkness was applied uniformly across all farms. Three commercial compound feeds were used during the fattening period, and their nutrient composition met the dietary requirements of the Ross 308 hybrid used in all fattening cycles. No differences in crude protein and metabolizable energy content among farms were observed. Feed and water were provided *ad libitum* throughout the fattening period, except for a withdrawal period of 10–12 hours prior to the planned transport of broilers to the slaughterhouse. Health management practices followed the recommendations of the official veterinary authority on all farms.

Five fattening cycles were conducted annually on each farm during each production year from 2022 to 2025, resulting in a total of 60 production cycles included in the analysis. The following data were obtained

from farm production records: number of chicks placed, fattening period (days), number of broilers delivered to the slaughterhouse, total live weight of broilers delivered (kg), total feed consumption (kg), and the number of broilers that died during each week of the fattening period. Based on these data, the following production parameters were calculated: feed conversion ratio (FCR, kg/kg), final body weight (kg), first-week mortality (%), total mortality (%), and the European Production Efficiency Factor (EPEF), as previously described by Szöllősi et al. (2021) and Biesek et al. (2022).

Statistical analysis included descriptive statistics (mean, minimum – Min, maximum – Max, Standard deviation – SD, coefficient of variation – CV) and one-way analysis of variance (ANOVA) to determine the effects of farm and production year on the analyzed production parameters. When significant effects were detected, pairwise comparisons were performed using Tukey's post hoc test at a significance level of $P < 0.05$. Phenotypic correlations between production parameters were assessed using Pearson correlation coefficient (r), with statistical significance set at $P < 0.05$. All statistical analyses were conducted using the R software environment.

RESULTS

Descriptive statistics of broiler production parameters across all farms and production years are presented in Table 1.

Table 1. Descriptive statistics of broiler production parameters across all production cycles

Parameters	Mean	Min.	Max.	SD	CV
Fattening period, days	40.20	36.00	45.00	2.49	6.19
Final body weight, kg	2.30	1.94	2.80	0.20	8.54
Feed conversion ratio	1.69	1.52	1.88	0.06	3.74
First-week mortality, %	1.71	0.50	4.80	0.83	48.29
Total mortality, %	3.92	1.79	8.00	1.35	34.37
EPEF	324.79	272.70	377.59	25.44	7.83

The average fattening period lasted 40.20 days, during which a mean final body weight of 2.30 kg was achieved with an average feed conversion ratio of 1.69. Mean first-week mortality was 1.71%, while total mortality per production cycle averaged 3.92%. The average EPEF value across all analyzed production cycles was 324.79. The highest variability was observed for first-week mortality and total mortality, as indicated by their high coefficients of variation (48.29% and 34.37%, respectively). In contrast, feed conversion ratio and fattening period showed the lowest coefficients of variation (3.74% and 6.19%, respectively).

Broiler production parameters across farms for the entire study period are presented in Table 2.

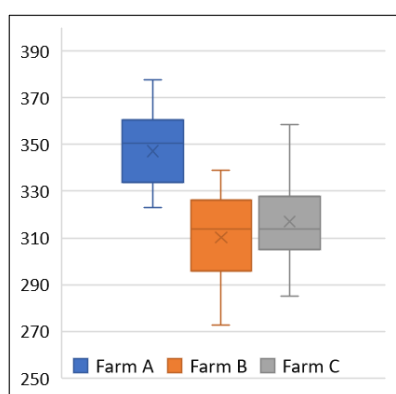
Table 2. Broiler production parameters according to farm

Farms	Farm A		Farm B		Farm C	
Parameters	Mean	SD	Mean	SD	Mean	SD
Fattening period, days	38.65 ^a	1.53	41.25 ^b	2.88	40.70 ^b	2.15
Final body weight, kg	2.28 ^a	0.14	2.33 ^a	0.26	2.28 ^a	0.18
Feed conversion ratio	1.64 ^a	0.06	1.73 ^b	0.05	1.71 ^b	0.04
First-week mortality, %	1.66 ^a	0.65	1.61 ^a	0.80	1.87 ^a	1.01
Total mortality, %	3.76 ^{ab}	0.80	4.55 ^a	1.68	3.45 ^b	1.22
EPEF	347.13 ^a	20.58	310.25 ^b	18.98	317.00 ^b	20.35

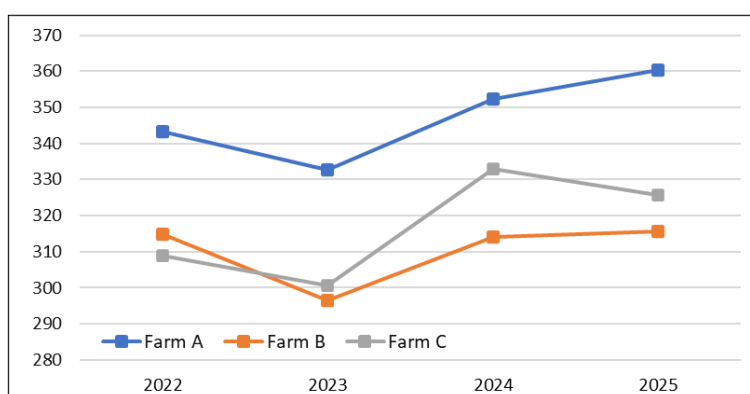
^{abc} Mean values in the same row followed by different letters indicate significant differences at $P < 0.05$

The fattening period was significantly influenced by farm, and it was shorter on Farm A (38.65 days) compared to Farm B (41.25 days) and Farm C (40.70 days) ($P < 0.05$). Final body weight ranged from 2.28 kg on Farms A and C to 2.33 kg on Farm B, with no statistically significant differences among farms ($P > 0.05$). Feed conversion ratio was significantly lower on Farm A (1.64) compared to Farms B and C (1.73 and 1.71, respectively) ($P < 0.05$). First-week mortality ranged from 1.61% on Farm B to 1.87% on Farm C and did not differ significantly between farms ($P > 0.05$). Total mortality was significantly lower on Farm C (3.45%) compared to Farm B (4.55%) ($P < 0.05$). EPEF was significantly higher on Farm A (347.13) compared to Farm B (310.25) and Farm C (317.00) ($P < 0.05$).

Figure 1 presents a box plot of EPEF values across the three farms for the entire study period. Farm A consistently showed the highest EPEF, while Farms B and C had lower and more variable values, consistent with the differences observed in Table 2.



Graph 1. EPEF across farms for entire study period (2022–2025)



Graph 2. EPEF across production years on farms during study period (2022–2025)

Broiler production parameters across production years during the study period (2022–2025) are presented in Table 3.

Table 3. Broiler production parameters according to production year (2022–2025)

Production years	2022		2023		2024		2025	
Parameters	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fattening period, days	39.60 ^a	2.64	39.40 ^a	2.10	40.80 ^a	2.78	41.00 ^a	2.20
Final body weight, kg	2.23 ^{bc}	0.18	2.17 ^c	0.12	2.38 ^{ab}	0.19	2.41 ^a	0.19
Feed conversion ratio	1.69 ^a	0.04	1.70 ^a	0.05	1.69 ^a	0.07	1.70 ^a	0.09
First-week mortality, %	1.52 ^a	0.62	1.58 ^a	0.72	1.63 ^a	0.72	2.12 ^a	1.11
Total mortality, %	3.55 ^a	1.17	4.49 ^a	1.76	3.82 ^a	0.85	3.82 ^a	1.39
EPEF	322.28 ^{ab}	19.61	309.95 ^b	23.85	333.06 ^{ab}	25.16	333.88 ^a	27.14

^{abc} Mean values in the same row followed by different letters indicate significant differences at $P < 0.05$

The fattening period did not differ significantly among production years, ranging from 39.40 days in 2023 to 41.00 days in 2025 ($P > 0.05$). Final body weight was significantly influenced by production year ($P < 0.05$), being lowest in 2023 (2.17 kg) compared to 2024 and 2025 (2.38 and 2.41 kg, respectively). Feed conversion ratio did not differ significantly among production years, with values of 1.69 in 2022 and 2024 and 1.70 in 2023 and 2025 ($P > 0.05$). First-week mortality ranged from 1.52% in 2022 to 2.12% in 2025, and non-significant differences were observed among production years ($P > 0.05$). Also, total mortality showed no significant differences, ranging from 3.55% in 2022 to 4.49% in 2023 ($P > 0.05$). EPEF was significantly influenced by production year ($P < 0.05$), with the highest value recorded in 2025 (333.88) compared to 2023 (309.95).

Figure 2 shows the temporal trends of EPEF for each farm from 2022 to 2025. Farm A maintained the highest EPEF values across all years, whereas Farm B exhibited lower values with slight fluctuations. Farm C showed intermediate values with a slight upward trend in the last year. These trends correspond with the results presented in Table 3.

The results of the correlation analysis among broiler production parameters, based on all production cycles in the study, are presented in Table 4.

Table 4. Pearson correlation matrix (r) of broiler production parameters

	Final body weight	Feed conversion ratio	First-week mortality	Total mortality	EPEF
Fattening period	0.75 *	0.37 *	-0.10 ^{NS}	0.10 ^{NS}	-0.19 ^{NS}
Final body weight		0.04 ^{NS}	0.09 ^{NS}	0.21 ^{NS}	0.43 *
FCR			-0.10 ^{NS}	0.02 ^{NS}	-0.75 *
First-week mortality				0.39 *	0.16 ^{NS}
Total mortality					-0.05 ^{NS}

* Correlation is significant ($P < 0.05$); ^{NS} Correlation is non-significant ($P > 0.05$).

Fattening period was significantly and positively correlated with final body weight ($r = 0.75$, $P < 0.05$) and feed conversion ratio ($r = 0.37$, $P < 0.05$). First-week mortality showed a significant and positive correlation with total mortality ($r = 0.39$, $P < 0.05$). EPEF was significantly and positively correlated with final body weight ($r = 0.43$, $P < 0.05$) and significantly and negatively correlated with feed conversion ratio ($r = -0.75$, $P < 0.05$). No significant phenotypic correlations were observed between EPEF and fattening period or mortality-related parameters ($P > 0.05$).

DISCUSSION

The average fattening period in the present study was 40.20 days and was significantly influenced by farm ($P < 0.05$), while no significant effect of production year was observed ($P > 0.05$). Broilers reared on Farm A had a significantly shorter fattening period (38.65 days) compared to those from Farm B (41.25 days) and Farm C (40.70 days), indicating that farm-specific management practices played a decisive role. These findings suggest that even under comparable regional and climatic conditions, there are differences in fattening period among farms.

Fattening periods have been reported in several European countries, 37.0-37.8 days in Germany (Junghans et al., 2022; Chibanda et al., 2024), 38.9 days in Turkey (Karaman et al., 2023), and 39.9-40.3 days in Hungary (Szöllősi et al., 2021), whereas notably longer fattening periods (45.3-47.0 days) have been observed in Greece (Dotas et al., 2021; Fotou et al., 2024). This variability reflects differences in production systems, target slaughter weights, and consumer preferences. In contrast to the present results, a previous study conducted on farms in the Banja Luka region during 2013-2014 production years did not confirm a significant influence of farm or production year on fattening period (Vekić et al., 2019), suggesting that recent changes in broiler production may have increased variability between farms over time.

The average final body weight recorded in this study was 2.30 kg and was significantly affected only by production year ($P < 0.05$), with the lowest value observed in 2023 (2.17 kg) compared to 2024 and 2025 (2.38 and 2.41 kg, respectively). The absence of a farm effect on final body weight indicates that, despite differences in fattening duration, farmers achieved relatively similar slaughter weights, likely reflecting standardized market requirements. In earlier research from the same region, neither farm nor year significantly influenced final body weight (Vekić et al., 2019), while similar stability across years was also reported on a Polish farm during the 2020-2023 period (Adaszyńska-Skwirzyńska et al., 2025).

The year effect observed in the present study may therefore be attributed to factors which are not directly related to the farms.

Globally, broilers fattened for 35-42 days typically reach final body weights between 2.0 and 2.2 kg (Abdalgilil, 2025), while within the European Union reported values range from 1.9 to 2.7 kg, depending on production objectives and consumer demand (Van Horne, 2018). The final body weights reported in the recent studies are: 2.25 kg in Turkey (Karaman et al., 2023), 2.30-2.69 kg in Germany (Junghans et al., 2022; Chibanda et al., 2024), 2.49-2.60 kg in Greece (Dotas et al., 2021; Fotou et al., 2024), 2.51 kg in Poland (Adaszyńska-Skwirzyńska et al., 2025), and 2.46-2.57 kg in Hungary (Szöllősi et al., 2021). Thus, both fattening period and final body weight in this study fall within these reference ranges, and representing an improvement compared to data from farms in the Banja Luka region during 2013-2014 production years (38.91 days, 2.07-2.13 kg, respectively) (Vekić et al., 2019; 2020).

Previous studies have demonstrated that fattening period and final body weight are influenced by a complex interaction of factors, including farm management, housing conditions, technological equipment, season, stocking density, flock size, and litter type (Campe et al., 2013; Szöllősi et al., 2021; Junghans et al., 2022). Although the farms included in the present study were similar with respect to housing characteristics and technical equipment, differences in flock management, and requirements of production integrators likely contributed to the observed variation in fattening duration and body weight. These results emphasize the importance of management in optimizing production efficiency.

Correlation analysis including all production cycles revealed a strong positive relationship between fattening length and final body weight ($r = 0.75$, $P < 0.05$), confirming that extended fattening periods are closely associated with higher slaughter weights. Similar relationships have been reported in previous studies conducted in Bosnia and Herzegovina (Mitrović et al., 2010; Vekić et al., 2019), as well as in Turkey (Karaman et al., 2023), South Korea (Kim et al., 2019), and India (Murugan et al., 2025).

The average feed conversion ratio (FCR) in this study was 1.69, which corresponds well with the global reference range for broilers fattened for 35-42 days (1.6-1.7), as reported by Abdalgilil (2025). This confirms that feed efficiency achieved under the production conditions of the present study was satisfactory and comparable to contemporary international standards. Moreover, the observed FCR represents a clear improvement compared to previously reported values of 1.79-1.80 recorded on farms in the Banja Luka region during 2013-2014 production years (Vekić et al., 2019; 2020), indicating a positive trend in production efficiency.

When compared with data from other countries, the FCR obtained in this study falls within the mid-range of reported values. Reported lower values were 1.54 in Poland (Adaszyńska-Skwirzyńska et al., 2025) and 1.57-1.64 in Hungary (Szöllősi et al., 2021), while higher values were 1.75 in Turkey (Karaman et al., 2023) and 1.74-1.81 in Greece (Dotas et al., 2021; Fotou et al., 2024). These differences likely reflect variation in production goals, climatic conditions, and feed formulations, highlighting that FCR should always be interpreted within the broader production context rather than as an isolated indicator.

In the present study, feed conversion ratio was significantly influenced by farm ($P < 0.05$), whereas production year had no significant effect ($P > 0.05$). This finding suggests that farm-specific factors played a more important role in determining feed efficiency than year-to-year variation. In contrast, earlier research conducted on farms in the Banja Luka region reported no differences in FCR between farms but did detect significant differences between production years (Vekić et al., 2019). Such discrepancies between studies underline the dynamic nature of broiler production systems, where farm and year related effects may shift over time. Consistent with the present results, no significant differences in FCR between production years were reported for the period 2020-2023 on a single Polish farm (Adaszyńska-Skwirzyńska et al., 2025).

Given that feed costs account for the largest proportion of variable costs in broiler production, even relatively small improvements in FCR can have substantial economic benefits (Adaszyńska-Skwirzyńska et al., 2025). Numerous studies have demonstrated that FCR is a multifactorial trait influenced by genotype, feed composition and quality, flock health status, and a wide range of management-related factors (Averós & Estevez, 2018; Baracho, 2019; Van Limbergen et al., 2020; Szöllősi et al., 2021). Although the farms included in the present study were broadly comparable in terms of housing characteristics and equipment, differences in management, and operational decisions may explain the observed farm effect on FCR.

Correlation analysis across all production cycles revealed a significant positive relationship between FCR and fattening length ($r = 0.37$, $P < 0.05$), indicating that longer fattening periods were associated with less efficient feed utilization. This relationship has been reported in previous studies from Bosnia and Herzegovina (Mitrović et al., 2010; Vekić et al., 2019), as well as from South Korea (Kim et al., 2019) and India (Murugan et al., 2025), and reflects the well-known decline in growth efficiency as broilers age. In contrast, no significant correlation was observed between FCR and final body weight ($r = 0.04$, $P > 0.05$). Under relatively standardized production conditions and within a narrow range of final body weights, feed efficiency appears to be more strongly driven by the duration of the fattening period than by absolute body weight at slaughter.

The average first-week mortality in this study was 1.71%, and it was not significantly influenced by either farm or production year ($P > 0.05$). A comparable first-week mortality of 1.82% was reported in Spain during the period 2015-2018 (Yerpes et al., 2020), whereas substantially lower values were observed in Germany (0.66%) (Junghans et al., 2022) and Hungary (0.93–0.98%) (Szöllősi et al., 2021). In the Banja Luka region, earlier studies also reported lower first-week mortality, averaging 0.71% (Vekić et al., 2020), indicating that the values observed in the present study are relatively elevated in comparison with both local and several European references.

First-week mortality is widely recognized as a indicator of day-old chick quality and early farm management quality. It is influenced by a complex interaction of factors related to the parent flock and hatchery conditions (e.g., breeder age, egg storage duration, incubation parameters), as well as chick genotype and sex, farm equipment, flock size, and seasonal conditions (Van Limbergen et al., 2020; Yerpes et al., 2020; Junghans et al., 2022). The first week of life represents a particularly critical period due to the transition from a highly controlled incubation environment to farm conditions, which are often more variable and may not fully meet the physiological requirements of hatched chicks (Yerpes et al., 2020). Consequently, the relatively higher first-week mortality observed in this study may be associated with day-old chick quality, as well as with farm preparation and management practices during the initial days after chick placement. Consistent with this interpretation, flocks exhibiting increased first-week mortality frequently experience higher total mortality throughout the fattening period (Campe et al., 2013; Van Limbergen et al., 2020; Junghans et al., 2022). This pattern was also confirmed in the present study, as a significant positive correlation was observed between first-week and total mortality ($r = 0.39$, $P < 0.05$).

The average total mortality in this study was 3.92%. At the global level, total mortality in broilers fattened for 35-42 days typically ranges from 4 to 5% (Abdalgil, 2025), although values up to 3% are considered acceptable under optimal production conditions (Baracho, 2019). Previously reported total mortality values in the Banja Luka region during 2013-2014 production years (4.07% and 3.24%) were relatively similar to those observed in the present study (Vekić et al., 2019; 2020), suggesting that no substantial improvement has been achieved in this parameter over time. In comparison with other countries, reported total mortality values were generally lower or comparable, including 2.38% in Poland (Adaszyńska-Skwirzyńska et al., 2025), 2.35-2.74% in Germany (Junghans et al., 2022; Chibanda et al., 2024), 2.91–3.96% in Greece (Dotas et al., 2021; Fotou et al., 2024), 3.52-4.59% in Hungary (Szöllősi et al., 2021), and 4.68% in Turkey (Karaman et al., 2023).

Total mortality is known to be affected by a wide range of factors, including season, litter quality and type, housing and environmental conditions, biosecurity level, and day-old chick quality (Campe et al., 2013; Averós & Estevez, 2018; Van Limbergen et al., 2020; Szöllősi et al., 2021). In the present study, total mortality did not differ significantly among production years ($P > 0.05$), but it was significantly influenced by farm ($P < 0.05$), indicating the importance of farm-specific management practices and environmental conditions. Apart from its association with first-week mortality, total mortality showed no significant correlation with other production traits. Similar findings regarding the lack of association between total mortality and fattening length or other performance parameters have been reported in the Banja Luka region (Vekić et al., 2019) and in South Korea (Kim et al., 2019). In contrast, a significant relationship between fattening length and total mortality was observed on a single farm over a five-year period in Bosnia and Herzegovina (Mitrović et al., 2010), suggesting that such associations may be influenced by farm-level conditions.

The relatively elevated mortality-related parameters observed in this study emphasize the need for more detailed investigation aimed at identifying their underlying causes. Given that mortality, together with feed conversion ratio, directly affects production efficiency and economic returns in broiler production (Karaman et al., 2023), improvements in early chick management, biosecurity, and farm-specific practices may represent an important opportunity to enhance overall flock performance.

The average European Production Efficiency Factor (EPEF) in this study was 324.79, indicating a high overall production efficiency. Previously, EPEF values above 190 (Karaman et al., 2023), 220 (Trentin et al., 2024), or 260 (Mavromati et al., 2018) were considered acceptable levels of satisfactory broiler performance. However, there is a trend toward increasing reference values, driven by continuous genetic progress in broiler strains and improvements in farm technology and management practices. In this context, it is noteworthy that all production cycles included in the present study achieved EPEF values above these commonly cited reference thresholds.

Compared with earlier data from the same region, the results of this study indicate a substantial improvement in EPEF, as previously reported values averaged 292.61 on farms in the Banja Luka region during the 2013-2014 production years (Vekić et al., 2019; 2020). In recent literature, several studies have reported lower EPEF values than those observed in the present study, including 209.15 (Bara et al., 2025), 218.8-292 (Mavromati et al., 2018), 295.10 (Dotas et al., 2021), 313.37 (Karaman et al., 2023), 314 (Fotou et al., 2024), and 322.22 (Atallah et al., 2021). Conversely, higher EPEF values have also been documented, such as 360.67 (Özel & Bozkurt, 2023), 355.39-394.55 (Szöllösi et al., 2021), 399 (Adaszyńska-Skwirzyńska et al., 2025), and 374-456 (Chibanda et al., 2024). These findings collectively suggest that the EPEF values achieved in this study reflect a relatively high level of production efficiency, while still leaving room for further optimization.

In the present study, EPEF was significantly influenced by both farm and production year ($P < 0.05$), highlighting the importance of farm-specific conditions and temporal variability in shaping overall production efficiency. In contrast, earlier research conducted on farms in the Banja Luka region during 2013–2014 did not detect significant differences in EPEF among farms or years (Vekić et al., 2019), while no year-to-year differences were observed between 2020 and 2023 on a single farm in Poland (Adaszyńska-Skwirzyńska et al., 2025). These discrepancies may reflect differences in farm capacity, flock management, or environmental conditions across studies.

Further improvements in EPEF on the farms included in this study will largely depend on continued optimization of the individual production parameters that constitute this aggregate index, namely fattening length, feed conversion ratio, and mortality. Achieving such improvements requires a comprehensive re-evaluation of the numerous factors that influence these parameters. Previous studies have shown that EPEF can be affected by chick sex, flock health status, type and functionality of feeding systems, feed composition, overall farm technology, and seasonal conditions (Van Limbergen et al., 2020; Szöllösi et al., 2021; Atallah et al., 2021; Özel & Bozkurt, 2023).

Correlation analysis in the present study revealed no significant association between EPEF and fattening length, which is consistent with findings reported by Mitrović et al. (2010) and Karaman et al. (2023). In contrast, an earlier study conducted in the Banja Luka region reported a negative correlation between fattening length and EPEF (Vekić et al., 2019), suggesting that the relationship between these variables may be influenced by farm-specific factors. Furthermore, EPEF showed a significant positive correlation with final body weight ($r = 0.43$, $P < 0.05$) and a strong negative correlation with feed conversion ratio ($r = -0.75$, $P < 0.05$), underscoring the dominant role of growth performance and feed efficiency in determining overall production efficiency. Identical patterns of correlation among these production parameters have also been reported on broiler farms in India (Murugan et al., 2025).

CONCLUSIONS

The present study evaluated broiler production performance on three commercial farms in the Banja Luka region during the period 2022–2025. The results demonstrated that both farm and production year significantly influenced several key production parameters. The average fattening period was 40.2 days, while the average feed conversion ratio was 1.69, and both parameters were significantly affected by farm.

The average final body weight was 2.30 kg and was influenced only by production year, suggesting the presence of annual variability related to environmental or organizational factors. First-week mortality and total mortality averaged 1.71% and 3.92%, respectively, with only total mortality showing a significant farm effect. The European Production Efficiency Factor averaged 324.79 and was significantly influenced by both farm and production year. Correlation analysis revealed that fattening period was positively associated with final body weight, feed conversion ratio was positively correlated with fattening period and negatively correlated with EPEF, while final body weight showed a positive association with EPEF. Overall, the studied farms achieved production results comparable to international benchmarks, with noticeable improvements in feed efficiency and EPEF compared to historical data from the region. These findings highlight that broiler production efficiency represents a complex trait influenced by farm management and annual variation. Continuous monitoring of key production indicators and targeted management interventions remain essential for optimizing broiler performance in commercial farm conditions.

REFERENCE

- Abdalgilil, F. S. (2025). A comparative economic analysis of broiler production costs: Selected Arab countries, Turkey, and the United States compared to global standards. *World Journal of Pharmaceutical Sciences*, 13(04), 43-59. <https://doi.org/10.54037/WJPS.2022.100905>
- Adaszyńska-Skwirzyńska, M., Konieczka, P., Buclaw, M., Majewska, D., Pietruszka, A., Zych, S., & Szczerbińska, D. (2025). Analysis of the production and economic indicators of broiler chicken rearing in 2020–2023: A Case study of a Polish farm. *Agriculture*, 15(2), 139. <https://doi.org/10.3390/agriculture15020139>
- Atallah, S. T., El-Ktany, E. M., & Ragab, E. M. (2021). Evaluation of the economic impact of strain, housing systems and season on commercial broiler performance and profitability under Egyptian condition. *Alexandria Journal of Veterinary Sciences*, 69 (2), 39-48. <https://doi.org/10.5455/ajvs.69235>
- AVEC. (2025). *Annual report*, 2025. <https://avec-poultry.eu/wp-content/uploads/2025/08/07394-AVEC-annual-report-2025.pdf>
- Averós, X., & Estevez, I. (2018). Meta-analysis of the effects of intensive rearing environments on the performance and welfare of broiler chickens. *Poultry Science*, 97(11), 3767-3785. <https://doi.org/10.3382/ps/pey243>
- Baéza, E., Guillier, L., & Petracci, M. (2022). Review: Production factors affecting poultry carcass and meat quality attributes. *Animal*, 16(1), 100331. <https://doi.org/10.1016/j.animal.2021.100331>
- Bara, Y., Baa, A., & Bir, A. (2025). Typology of broiler farms in M'sila region (Algeria): Zootechnical-economic performance. *Advances in Animal and Veterinary Sciences*, 13(8), 1681-1688. <https://dx.doi.org/10.17582/journal.aavs/2025/13.8.1681.1688>
- Baracho, M. S., Nääs, I. A., Lima, N. D. S., Cordeiro, A. F. S., & Moura, D. J. (2019) Factors affecting broiler production: A meta-analysis. *Braz. J. Poult. Sci.*, 21, 3, <https://doi.org/10.1590/1806-9061-2019-1052>
- Biesek, J., Banaszak, M., Wlaźlak, S., & Adamski, M. (2022). The effect of partial replacement of milled finisher feed with wheat grains on the production efficiency and meat quality in broiler chickens. *Poultry Science*, 101(5), 101817. <https://doi.org/10.1016/j.psj.2022.101817>
- Campe, A., Koesters, S., Niemeyer, M., Klose, K., Ruddat, I., Baumgarte, J., & Kreienbrock, L. (2013). Epidemiology of influences on the performance in broiler flocks — A field study in Germany. *Poultry Science*, 92(10), 2576-2587. <https://doi.org/10.3382/ps.2013-03207>
- Chibanda, C., Thobe, P., Almadani, M. I., van Horne, P., Deblitz, C., & Wieck, C. (2024). Improving broiler farm competitiveness in Ghana and Senegal: insights from comparative analysis with Germany and the Netherlands. *Agriculture & Food Security*, 13(65). <https://doi.org/10.1186/s40066-024-00519-8>
- Choi, J., Kong, B., Bowker, B. C., Zhuang, H., & Kim, W. K. (2023). Nutritional strategies to improve meat quality and composition in the challenging conditions of broiler production: A review. *Animals*, 13(8), 1386. <https://doi.org/10.3390/ani13081386>

- Dotas, V., Gourdouvelis, D., Hatzizisis, L., Kaimakamis, I., Mitsopoulos, I., & Symeon, G. (2021). Typology, structural characterization and sustainability of integrated broiler farming system in Epirus, Greece. *Sustainability*, 13(23), 13084. <https://doi.org/10.3390/su132313084>
- Fotou, E., Moulasioti, V., Papadopoulos, G. A., Kyriakou, D., Boti, M. E., Moussis, V., Papadami, M., Tellis, C., Patsias, A., Sarrigeorgiou, I., Theodoridis, A., Lymberi, P., Tsiouris, V., Tsikaris, V., & Tsoukatos, D. (2024). Effect of farming system type on broilers' antioxidant status, performance, and carcass traits: An Industrial-scale production study. *Sustainability*, 16(11), 4782. <https://doi.org/10.3390/su16114782>
- Gržinić, G., Piotrowicz-Cieślak, A., Klimkowicz-Pawlas, A., Górny, R. L., Ławniczek-Wałczyk, A., Piechowicz, L., Olkowska, E., Potrykus, M., Tankiewicz, M., Krupka, M., Siebielec, G., & Wolska, L. (2023). Intensive poultry farming: A review of the impact on the environment and human health. *Science of the Total Environment*, 858(3), 160014. <https://doi.org/10.1016/j.scitotenv.2022.160014>
- Jalić, N., Vekić, M., Ostojić, A., & Mrdalj, V. (2023). Proizvodnja, promet i potrošnja pilećeg mesa u Bosni i Hercegovini. *Agroekonomika*, 52(98), 1-9.
- Junghans, A., Deseniş, L., & Louton, H. (2022). Data evaluation of broiler chicken rearing and slaughter - An exploratory study. *Frontiers in Veterinary Science*, 9, 957786. <https://doi.org/10.3389/fvets.2022.957786>
- Karaman, S., Taşcıoğlu, Y., & Bulut, O. D. (2023). Profitability and cost analysis for contract broiler production in Turkey. *Animals*, 13(13), 2072. <https://doi.org/10.3390/ani13132072>
- Kim, G. W., Kim, J. H., Kim, H. Y., Kim, B. K., Park, H. B., Choe, J., Kim, & J. H. (2019). Analysis of marketing performances according to raising environment in broilers. *Korean Journal of Poultry Science*, 46(1), 25-30. <https://doi.org/10.5536/KJPS.2019.46.1.25>
- Mavromati, E., Sena, L., Gjeta, Z., & Mavromati, J. (2018). Assessing the economic efficiency in some broiler farms through the European Production Efficiency Factor (EPEF). *European Academic Research*, 6(9), 5354-5362.
- Mitrović, S., Pupavac, S., Đermanović, V., & Ostojić, Đ. (2010). Effect of duration fattening the production performance broilers chickens of hybrid Cobb 500. *Radovi sa 24. savetovanja agronoma, veterinara i tehnologa*, 16(3-4), 163-170.
- Murugan, M., Edwin, S.C., & Raghavan, A. (2025). Influence of economic parameters and season on broiler performance efficiency factor (BPEF) in commercial farming. *Indian Journal of Veterinary and Animal Sciences Research*, 54(1), 88-95.
- Neeteson, A. M., Avendaño, S., Koerhuis, A., Duggan, B., Souza, E., Mason, J., Ralph, J., Rohlf, P., Burnside, T., Kranis, A., & Bailey, R. (2023). Evolutions in commercial meat poultry breeding. *Animals*, 13(19), 3150. <https://doi.org/10.3390/ani13193150>
- Özel, F., & Bozkurt, Z. (2023). The effect of season on the performance, health, and welfare of broilers. *Harran University Journal of the Faculty of Veterinary Medicine*, 12(2), 196-201. <https://doi.org/10.31196/huvfd.1335400>
- Roskam, J. L., Oude Lansink, A. G. J. M., & Saatkamp, H. W. (2020). The relation between technical farm performance and antimicrobial use of broiler farms. *Poultry Science*, 99(3), 1349-1356. <https://doi.org/10.1016/j.psj.2019.10.054>
- Salihbašić, E., Vaško, Ž., Bašić, M., & Ahmetović, M. (2015). Trendovi u proizvodnji i potrošnji mesa peradi u svijetu i Bosni i Hercegovini. *Agroznanje*, 16(4), 559-570. <https://doi.org/10.7251/AGRSR1504559S>
- Szöllösi, L., Béres, E. & Szűcs, I. (2021). Effects of modern technology on broiler chicken performance and economic indicators – a Hungarian case study. *Italian Journal of Animal Science*, 20(1), 188-194. <https://doi.org/10.1080/1828051X.2021.1877575>
- Trentin, A., Talamini, D. J. D., Coldebella, A., Pedroso, A. C., & Gomes, T. M. A. (2024). Technical and economic performance favours fully automated climate control broiler housing. *British Poultry Science*, 66(1), 63-70. <https://doi.org/10.1080/00071668.2024.2394182>

- Van Horne, P. L. M. (2018). Competitiveness of the EU poultry meat sector, base year 2017: international comparison of production costs. Wageningen Economic Research, Wageningen.
- Van Limbergen, T., Sarrazin, S., Chantziaras, I., Dewulf, J., Ducatelle, R., Kyriazakis, I., McMullin, P., Méndez, J., Niemi, J. K., Papasolomontos, S., Szeleszczuk, P., Van Erum, J., & Maes, D. (2020). Risk factors for poor health and performance in European broiler production systems. *BMC Veterinary Research*, 16(1), 287. <https://doi.org/10.1186/s12917-020-02484-3>
- Vekić, M., Klincov, Ž., Jotanović, S., & Savić, Đ. (2019). Basic production parameters of small capacity broiler farms in the Region of Banja Luka. *Agro-knowledge Journal*, 20(4), 187-196. <https://doi.org/10.7251/AGREN1904187V>
- Vekić, M., Klincov, Ž., Jotanović, S., & Savić, Đ. (2020) Production results of broiler chicken farms of different capacities in region of Banja Luka. *IX International Symposium on Agricultural Sciences AgroReS 2020, Proceedings*, 163-170.
- Yerpes, M., Llonch, P., & Manteca, X. (2020). Factors associated with cumulative first-week mortality in broiler chicks. *Animals*, 10(2),310. <https://doi.org/10.3390/ani10020310>
- Zenunović, A., & Glavić, M. (2017). The situation in the poultry sector in Bosnia and Herzegovina. *International Journal of Advances in Agricultural Science and Technology*, 4(12), 1-6.

Digital technologies in dairy farming: Implications for efficiency, sustainability, and animal welfare in North Macedonia and Croatia

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Abstract

The increasing integration of digital technologies in dairy farming presents a transformative opportunity for enhancing productivity, sustainability, and animal welfare. This study examines the application of advanced digital tools and precision livestock farming (PLF) systems on dairy farms in Croatia and North Macedonia, with a focus on their impact on operational efficiency, milk quality, and herd management. Data were collected from multiple farms, including case studies in the Pelagonia region of North Macedonia and the Slavonia region of Croatia, supplemented by official statistics and industry reports. The analysis encompasses the use of automated milking systems, robotic milking, precision feeding, health monitoring devices, biogas production, and digital farm management software. Results indicate that farms employing these technologies demonstrate significant improvements in productivity and resource management, including optimized feeding, early disease detection, and enhanced environmental sustainability. In Croatia, digital systems enable nationwide monitoring of milk composition and cow health, contributing to more precise nutritional management and improved milk quality. In North Macedonia, farms in Pelagonia exhibit higher adoption rates of advanced digital technologies compared to other regions, reflecting the role of regional infrastructure, training, and investment in technology implementation. The findings highlight that digitalization in dairy farming supports economic viability, reduces labor dependency, and enhances overall animal welfare. Furthermore, the adoption of renewable energy solutions and data-driven decision-making facilitates sustainable practices and long-term resilience of dairy farms. This study underscores the critical role of precision technologies in addressing labor shortages, optimizing herd management, and fostering environmentally sustainable dairy production. The results provide evidence for policymakers, farm managers, and stakeholders regarding the benefits of integrating digital solutions into dairy farming operations, thereby contributing to the modernization and competitiveness of the dairy sector in Southeast Europe.

Keywords: dairy farming, digital technologies, precision livestock farming, sustainability, animal welfare

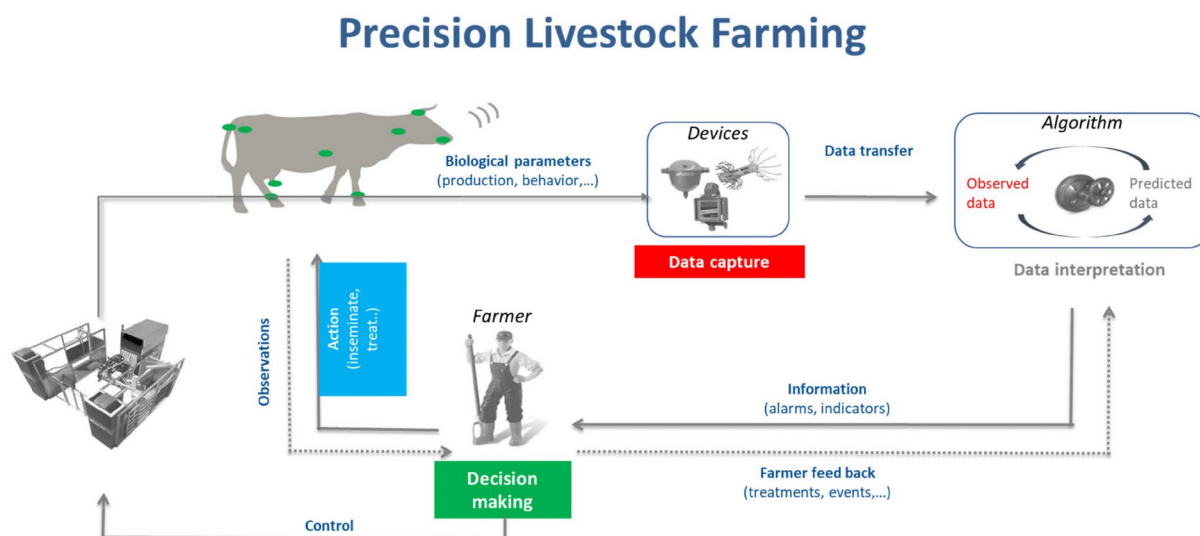
INTRODUCTION

In many European countries, cattle farming constitutes a significant portion of total animal production, contributing approximately 70% of the total income in the sector. This production is primarily categorized into two main segments: dairy, which focuses on the production of milk and related products, and beef, which pertains to the rearing of cattle for meat. The integration of digital technologies in livestock farming is becoming increasingly crucial, particularly in light of the current

labor shortages that affect the agricultural sector as a whole. Innovations such as precision farming, automated milking systems, and advanced data analytics are transforming traditional practices, enabling farmers to optimize their operations. As technology continues to advance, the potential for digital solutions in both dairy and beef production to revolutionize the industry is immense. These innovations not only promise to enhance sustainability by reducing waste and improving resource management but also significantly boost efficiency and economic viability. The adoption of such technologies can lead to better animal welfare, higher quality products, and increased profitability for producers, thereby reshaping the landscape of the dairy and beef industries for the future (Agroskills, 2023; Stricker, 2003). This paper aimed to analyse the fundamental principles of digital technologies in dairy farming while acknowledging the increasing necessity of precision technologies in modern livestock production. Additionally, it presents case studies from dairy farms in Croatia and North Macedonia, illustrating the successful implementation of these advanced technologies and their impact on production efficiency, animal health, and overall farm sustainability.

PRINCIPLES OF DIGITAL TECHNOLOGIES IN DAIRY FARMING

Digital dairy production encompasses the application of advanced technologies and data-driven systems to enhance and optimize dairy farming operations. This approach integrates Internet of Things (IoT) devices, sensors, artificial intelligence (AI), machine learning, and data analytics to enable real-time monitoring, precise management, and continuous improvement of key aspects of dairy farming, including animal health, milk production, and overall sustainability (Kleen and Guatteo, 2023). The implementation of digital technologies in dairy farming significantly enhances operational efficiency by automating routine tasks and optimizing resource utilization, thereby reducing labor requirements and increasing overall productivity. Continuous health and behavioral monitoring of dairy cattle enables early detection of potential health issues, contributing to improved animal welfare through timely intervention and better care. Additionally, digitalization supports sustainable farming practices by optimizing the use of feed, water, and energy resources, ultimately reducing the environmental footprint of dairy production. From an economic perspective, data-driven decision-making facilitates the identification of inefficiencies, allowing for more precise resource allocation and long-term cost reductions. By integrating digital technologies into dairy farming systems, farmers can achieve greater precision in herd management, enhance productivity, and contribute to more sustainable and economically viable dairy production. Basic functioning of precision livestock farming system on dairy cattle farm is presented on Picture 1.



Picture 1. Presentation of PLF system on a dairy farm (Kleen and Guatteo, 2023)

There are several key components that contribute to the optimization of production processes, animal welfare, and resource management through the application of digital tools and data-driven decision-making. One of the fundamental aspects of digital dairy production is the use of *smart sensors for continuous monitoring of animal health and behavior*. Wearable devices, such as smart collars and ear tags, collect real-time data on body temperature, heart rate, rumination patterns, and activity levels. These data enable the early detection of diseases and other health-related issues, allowing for timely intervention and improved animal welfare.

Automated milking systems (AMS), commonly known as robotic milking machines, facilitate the milking process without direct human intervention. These systems monitor milk yield, quality, and milking frequency, ensuring optimal efficiency while reducing labor requirements and enhancing overall herd management. *Precision feeding technologies* enable the optimization of feed rations based on real-time data, ensuring that cows receive the necessary nutrients to maintain health and productivity while minimizing feed waste and reducing costs. Smart feeding systems adjust feed portions according to the specific needs of individual cows, enhancing efficiency and sustainability.

Farm management software provides dairy farmers with comprehensive tools to oversee various aspects of farm operations, including animal health monitoring, production data analysis, and financial management. These digital platforms analyze trends, predict outcomes, and support informed decision-making, improving farm productivity and economic viability.

Artificial intelligence (AI) and machine learning play a crucial role in digital dairy farming by processing large datasets from sensors, cameras, and other sources to detect patterns and optimize farm management strategies. AI-driven models can predict milk production trends, assess breeding efficiency, and identify potential health risks, thereby improving productivity and disease prevention measures. *Supply chain and logistics optimization* is another critical component of digital dairy production, ensuring efficient milk collection, transportation, and distribution. Digital tracking technologies monitor milk quality, temperature, and delivery routes in real time, improving product quality and minimizing losses.

Sustainability in dairy farming is significantly enhanced through digitalization by optimizing water and feed usage, improving energy efficiency, and implementing automated manure management systems. Precision irrigation and emissions monitoring technologies contribute to reducing the environmental footprint of dairy production, aligning with sustainability objectives.

Traceability and transparency in the dairy supply chain are facilitated through blockchain and other digital tracking technologies. These systems enable complete traceability of milk from farm to consumer, ensuring transparency in production practices and enhancing food safety standards. Consumers can access detailed information regarding the origin of dairy products, farming methods, and quality assurance processes, reinforcing trust in the dairy industry. Finally, by integrating these digital technologies, dairy farming operations can achieve higher efficiency, improved animal health, enhanced sustainability, and greater transparency, ultimately contributing to the advancement of modern dairy production systems (EPRS, 2016).

DIGITALIZATION OF DAIRY CATTLE FARMS IN NORTH MACEDONIA

One of the pressing challenges facing the agricultural sector in the RNM country is the alarming trend of an ageing workforce. Currently, only 4% of farm owners are under the age of 35, while a substantial 62% are over 55 years old (SCS, 2023). This demographic imbalance poses significant threats to the sector's sustainability and future growth, as it reveals a critical shortage of young, motivated farmers willing to enter the field. Furthermore, the agricultural workforce is characterized by a low level of formal education. Many farmers have completed only primary or secondary education, with a strikingly small percentage possessing any formal training in agriculture. This educational gap severely impedes the advancement of modern techniques and practices within the sector. The deficiency in formal education, combined with a lack of training and managerial expertise, serves as a significant barrier to much-needed modernization, restructuring, and innovation in agricultural practices. An additional concern is the inadequate transfer of technology and innovation, primarily due to weak relationships and

collaborations among key institutions within the agricultural system. While some academic and scientific institutions own agricultural land, they often lack the necessary support and resources to implement substantial applied research projects, which stifles opportunities for technology demonstration and advancement.

In today's fast-paced agricultural landscape, modern farming necessitates robust support mechanisms that encourage the swift exchange of information. This requires a comprehensive approach, emphasizing interconnected systems that facilitate seamless communication among producers, researchers, and policymakers. By broadening access to essential resources and information, the agricultural sector can better equip itself to meet contemporary challenges and capitalize on new opportunities for growth and sustainability (Digital Austria, 2020).

Digital infrastructure in North Macedonia

The digital infrastructure of the country displays a complex situation. Fixed broadband subscriptions remain limited, with approximately 20 subscriptions per 100 inhabitants. In contrast, mobile services have attained comparatively high penetration rates, with nearly the entire population having access to 3G and LTE networks. Furthermore, around 79% of the population utilizes the Internet. Nevertheless, digital skills among the active workforce are considered to be at a moderate level, indicating significant potential for enhancement.

Digital skills and ICT in agriculture in North Macedonia

Macedonian farmers demonstrate a considerable proficiency with basic digital tools, including smartphones (55%), computers (70%), and the Internet (60%). These skills indicate a strong foundation for the adoption of digital technologies in agriculture. However, farmers exhibit limited awareness and knowledge of advanced ICT-based technologies, such as automated systems, GPS, GIS, and precision agriculture tools. To fully capitalize on digital innovations in agriculture, there is a clear need for enhanced capacity building and awareness initiatives.

Regarding the total cattle population, from the year 2011 to 2024, North Macedonia has seen a decline in its cattle numbers, decreasing from 268,000 to 130,000 heads—approximately half of the original total. Furthermore, according to official statistical data for the year 2024, the cattle population in the Republic of North Macedonia stands at 131,200, comprised of 2% domestic Busha cattle, 39% Holstein, and 52% crossbreeds (FVA, 2024). In the realm of organic cattle production, in the year 2021, out of a total of 145,299 heads, there were 9,752 in organic farming. By 2023, from 128,467 total heads, the number of cattle in organic production has decreased to 7,528 (FVA, 2024; SCS, 2024).

Case study 1: Practical application of digital technology on dairy farms in the RNM (Pelagonia region)

Recent developments in digital dairy farming have emerged on several dairy farms within the Pelagonia agricultural region of North Macedonia. This region is widely recognized as the optimal area for dairy farming in the country, owing to its favorable climatic conditions and fertile pastures. Recently, two dairy farms in Pelagonia have integrated advanced smart electricity technologies aimed at enhancing their operational efficiency. The technologies implemented include:

- *Robot Milking Systems*: Automated milking machines that use robotics to optimize the milking process, improving efficiency and allowing for flexible milking schedules.
- *Digital Monitoring Technology*: Innovative systems for assessing milk quality and safety, including automated collection and analysis of milk samples to ensure compliance with health standards.
- *Smart Tractor Software*: Enhanced software for tractors equipped with GPS navigation for precision crop production, facilitating efficient land management and resource application.
- *Automated Feed Centre*: A sophisticated center dedicated to the formulation and preparation of daily feed rations for beef cattle, utilizing technology to optimize nutritional values.
- *Feeding Machinery for Dairy Cows*: Advanced machinery designed to create total mix rations, ensuring that dairy cattle receive a balanced diet for optimal milk production.

- *Bovine Health Monitoring Boluses*: Ingestible devices placed in the stomachs of dairy cows to provide real-time insights into digestion and overall health, enabling early detection of diseases, reducing antibiotic usage, streamlining reproductive processes, and enhancing herd welfare.
- *Lacto Freezers*: High-efficiency storage systems designed for the cooling and preservation of milk, maintaining quality until it reaches processing facilities.
- *Smart Cleaning Equipment*: Automated systems for washing and sanitizing milking equipment, ensuring hygienic conditions and compliance with health regulations.
- *Farm Management Software*: Comprehensive platforms enabling farmers to manage reproductive cycles, nutritional needs, and selection processes for dairy cows, driving efficiency and productivity.
- *Wearable Technology for Cattle*: Pedometers and smart collars equipped with sensors to monitor body activity, temperature, reproductive status, and behavioural patterns, providing invaluable data for herd management.
- *Selective Breeding Strategies*: The utilization of sexed semen for cow insemination to preferentially produce female calves, optimizing herd productivity and profitability.
- *Biogas Production Facility*: One farm has developed a biogas plant that converts cattle manure into renewable energy, contributing to sustainable farming practices.
- *Surveillance Cameras*: Strategically placed cameras in barn environments to monitor cattle activity, ensuring welfare and safety.
- *Modern Cattle Barns*: Construction of state-of-the-art barns designed with climate control systems to ensure optimal living conditions for cattle, enhancing comfort and productivity.
- *Electric Fencing*: Implementation of 12-volt electric fencing systems for effective grazing management and to protect livestock from predators.
- *Manure Handling Equipment*: Electric machines equipped with scrapers for efficient manure removal from barns, contributing to a cleaner and healthier farming environment.
- *Solar Energy Systems*: Installation of solar panels for the generation of renewable energy to power various farm operations, reducing reliance on external energy sources.
- *Wind Energy Stations*: Utilization of wind turbines for on-site electricity generation, further promoting sustainable energy practices in agriculture.
- *Digital Meteorological Stations*: Installation of weather monitoring stations to provide real-time data on climatic conditions, assisting farmers in making informed decisions about planting and harvesting.
- *Irrigation Systems*: Advanced "drop by drop" irrigation techniques that enhance water efficiency, ensuring crops receive optimal hydration without waste.
- *Cordless Internet Connectivity*: Provision of wireless internet across the farm, facilitating the use of smart technologies and enhancing communication.
- *Electric Wells*: Electrically controlled systems for efficient water supply management throughout the farm, supporting irrigation and livestock hydration.
- *Drones for Monitoring*: Deploying drones for aerial observation of cattle movements across pastures, allowing for better management and supervision of grazing patterns.

Furthermore, in the agricultural region of Pelagonia, there are over 20 family-owned dairy farms that have embraced modern advancements by incorporating automatic milking machines, though these systems do not yet include fully autonomous robots. These farms are also utilizing smart digital technologies, enhancing their operational efficiency and productivity. In contrast, the remaining seven agricultural regions in North Macedonia continue to see a predominance of small-scale dairy farmers who have yet to fully embrace these innovations. In these areas, the use of smart electrical technologies is notably limited. While most farms are equipped with basic lacto-freezers and traditional milking machines, few have implemented more advanced renewable energy solutions, such as solar panels for self-sufficient electricity generation. This disparity highlights the potential for growth and modernization among smaller dairy operations outside of Pelagonia.

DIGITALIZATION OF DAIRY CATTLE FARMS IN CROATIA

In Croatia, the digitalization of dairy cattle farms, particularly in the context of milk production, utilizes precise estimations of dairy cow traits based on milk control results. The evaluation of milk composition, such as the relationship between milk fat and protein content, is a key indicator for assessing both the metabolic and nutritional status of dairy herds. Specifically, the ratio of milk fat to protein (M/B ratio) plays a crucial role in determining the health and nutrition of cows.

The optimal M/B ratio is between 1.1 and 1.5, and any significant deviation from this range can indicate health or nutritional issues. For example, an M/B ratio higher than 1.5 is often linked to conditions such as reduced appetite, ketosis, or mastitis, which are typically associated with cows with high milk fat content at the beginning of lactation (Duffield, 2004; Eicher, 2004). On the other hand, an M/B ratio lower than 1.1 usually reflects inadequate feeding practices, often a high proportion of concentrated feed or abrupt dietary changes (Duffield, 2004; Babnik et al., 2004).

Additionally, the content of lactose, urea, and somatic cells in milk are valuable indicators for diagnosing metabolic disorders and evaluating nutritional status. For instance, low lactose levels correlate with high somatic cell counts, which are a sign of mastitis, a common issue in dairy farming (Babnik et al., 2004; Eicher, 2004). Urea levels in milk are also linked to protein intake and overall nutritional balance (Duffield, 2004). The ability to track these parameters through digital systems allows farmers to quickly identify issues and adjust improve herd health and milk quality.

This digital approach is implemented for the entire population of dairy cows in Croatia, ensuring that these monitoring systems are applied on a national scale. The digitalization of dairy farms in Croatia, therefore, enables more precise monitoring of milk quality and cow health, which is essential for optimizing production and ensuring sustainable practices in dairy farming. Through the integration of these technologies, farmers can enhance their ability to manage herd health, reduce the risk of diseases, and improve the overall efficiency of dairy operations.

Furthermore, in Croatia, on a significant number of dairy farms, automated milking and robotic milking systems are being actively utilized. These systems offer substantial improvements in efficiency and labor management, reducing the time and effort required for manual milking. The adoption of robotic milking also facilitates better monitoring of individual cow performance, including milk production, health, and behavior, enabling more precise management of the herd. Additionally, cooling systems equipped with fans and humidifiers are widely employed to enhance the welfare of dairy cows, particularly in warmer months. These systems help regulate temperature and humidity in the barn, providing a more comfortable environment for the cows, which in turn can improve milk yield and overall health. Effective cooling is particularly crucial during the summer, as heat stress can negatively affect both milk production and reproduction. Furthermore, precision feeding techniques are increasingly being adopted in Croatian dairy farming. These methods allow for tailored nutrition based on the specific needs of individual cows or groups within the herd. Precision feeding systems ensure that cows receive the right amount of nutrients, optimizing milk production, improving animal health, and reducing waste. By using technologies that monitor intake and adjust feed rations accordingly, farms can enhance efficiency and sustainability. These advancements in dairy farm management not only contribute to better productivity but also to improved animal welfare, making Croatian dairy farms more competitive and environmentally responsible.

Case study 2: Practical application of digital technology on dairy farms in the Croatia (Slavonia region)

In the Slavonia region of Croatia, more than 10 dairy farms have adopted various digital technologies that enhance the precision and efficiency of dairy farming practices. These farms are utilizing automated milking systems, robotic milking, and advanced cooling systems equipped with fans and humidifiers. These technologies not only improve milk production but also optimize animal health and welfare by offering a more controlled environment. In addition to the automated milking systems, the region has seen the implementation of precision feeding technologies, enabling better nutritional management for the dairy herds. These technologies allow for more accurate feed formulation based on individual animal needs, optimizing milk yield and quality while minimizing feed waste. Moreover, the use of advanced

land management systems is becoming increasingly prevalent. These systems enhance crop production, improve soil health, and contribute to more sustainable farming practices. This integrated approach to farming ensures that the environmental impact is minimized while maximizing productivity on both the crop and dairy sides.

CONCLUSION

This study demonstrates that the integration of digital technologies in dairy farming substantially enhances productivity, efficiency, and sustainability. Precision livestock farming tools, including automated milking, health monitoring, precision feeding, and digital management platforms, allow for more accurate herd management, early disease detection, and optimized resource use. Case studies from Croatia and North Macedonia highlight that farms adopting these technologies achieve higher operational efficiency, improved milk quality, and better animal welfare compared to traditional practices. Additionally, renewable energy systems and data-driven decision-making support environmentally sustainable operations and long-term farm resilience. Given the challenges of labor shortages, aging farm populations, and fragmented farm structures, digitalization emerges as a critical solution for ensuring the competitiveness and sustainability of the dairy sector. Overall, the study underscores that precision technologies are not only a tool for immediate operational improvements but also a strategic investment in the future viability of dairy farming in Southeast Europe.

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REFERENCES

- Brickell, J. S., Bourne, N., McGowan, M. M., & Wathes, D. C. (2009). Effect of growth and development during the rearing period on the subsequent fertility of nulliparous Holstein-Friesian heifers. *Theriogenology*, 72, 408–416. <https://doi.org/10.1016/j.theriogenology.2009.03.015>
- AgriSkills 4.0 e-Learning Platform (2023). *Modules*. <https://training.agriskills40.com/modules/>
- Babnik, D., Verbič, J., Podgoršek, P., Jeretina, J., Perpar, T., Logar, B., Sadar, M., & Ivanovič, B. (2004). *Priročnik za vodenje prehrane krav molznic ob pomoči rezultatov mlečne kontrole*, Kmetijski inštitut Slovenije, Ljubljana.
- Digital Austria (2020). Digitalization report: *Now for Tomorrow - Digitalization for growth and futureproofing*. https://www.digitalaustria.gv.at/digitalisierungsbericht_en.html
- Duffield, T.F. (2004). *Monitoring strategies for metabolic disease in transition dairy cows*. 23rd World Buiatrics Congress, Quebec City, Canada.
- Eicher, R. (2004). *Evaluation of the metabolic and nutritional situation in dairy herds: Diagnostic use of milk components*. 23rd World Buiatrics Congress, Quebec City, Canada.
- European Parliamentary Research Service, EPRS (2016). *Precision agriculture and the future of farming in Europe. Scientific Foresight Study*. [https://www.europarl.europa.eu/RegData/etudes/STUD/2016/581892/EPRS_STU\(2016\)581892_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/STUD/2016/581892/EPRS_STU(2016)581892_EN.pdf)
- Food and Veterinary Agency (FVA). Registries. <https://fva.gov.mk/en/registries>
- Gantner, V. (2022). *Digitalizacija u mesnim stadima*. Zbornik predavanja, 17. savjetovanje uzgajivača goveda u Republici Hrvatskoj, Sveti Martin na Muri, 06-07.04.2022, str. 119-127.
- Giesler, S. (2018). *Digitalization in agriculture –from precision farming to farming 4.0*. Bioeconomy BW. <https://www.biooekonomie-bw.de/en/articles/dossiers/digitisation-in-agriculture-from-precision-farming-to-farming-40>.

- Hegde, P. (2021). *Precision Agriculture: How is it different from Smart Farming?* Cropin Technology. <https://www.cropin.com/blogs/smart-farming-vs-precision-farming-systems>
- Innovation Farm (2025). Farming for future. <https://www.innovationfarm.at/>
- Kleen, J.L., & Guatteo, R. (2023). Precision Livestock Farming: What Does It Contain and What Are the Perspectives? *Animals* 2023, 13, 779. <https://doi.org/10.3390/ani13050779>
- Kurth, T., Subei, B., Plötner, P., Bünger, F., Havermeier, M., & Krämer, S. (2023). The Case for Regenerative Agriculture in Germany— and Beyond, March 2023, NABU (Nature and Biodiversity, Conservation Union). https://www.nabu.de/imperia/md/content/nabude/landwirtschaft/230323-the_case_for_regenerative_agriculture_longversion-engl.pdf.
- Stricker, S. (2003). Situation of Agricultural Information and Communication Technology (ICT) in Germany. Research Gate, https://www.researchgate.net/publication/228987253_Situation_of_Agricultural_Information_and_Communication_Technology_ICT_in_Germany
- Министерство за земјоделство, шумарство и водостопанство - MZSV (2021). *Национална стратегија за земјоделството и руралниот развој за периодот 2021-2027 Година*. <https://ipard.gov.mk/wp-content/uploads/2021/02/Национална-стратегија-за-земјоделство-и-рурален-развој-2021-2027.pdf>

Regional variability in milk production, milk urea nitrogen, and ammonia emissions in dairy cows in Croatia

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Abstract

This study aimed to evaluate regional variability in milk production traits, milk urea, milk urea nitrogen (MUN), and ammonia emissions in dairy cows in Croatia. The analysis was based on 3,953,637 test-day records of Holstein cows collected between 2005 and 2022 within the official milk recording system. Farms were grouped into three breeding regions: Central, Eastern, and Mediterranean Croatia. The results showed clear regional differences in production traits. Eastern Croatia achieved the highest average daily milk yield (24.39 kg), while Central Croatia had the lowest (20.59 kg) and the highest variability. Mediterranean Croatia showed intermediate milk yield but lower milk fat and protein content. Considerable variability in all traits was observed across regions. Nitrogen-related parameters also differed among regions. The highest values of milk urea (24.19 mg/dL), MUN (11.13 mg/dL), and ammonia emissions (80.97 g/cow/day) were recorded in Mediterranean Croatia, while the lowest values were observed in Central Croatia. Eastern Croatia showed intermediate but relatively consistent values. The results suggest that regional differences in production performance and nitrogen metabolism may be associated with variations in feeding practices, production intensity, and management systems. Higher urea and MUN levels were associated with increased ammonia emissions, indicating lower nitrogen utilization efficiency. These findings emphasize the importance of region-specific management and feeding strategies in improving production efficiency and reducing environmental impacts in dairy production.

Keywords: Holstein cows, milk urea, milk urea nitrogen, ammonia emissions, Croatian dairy regions

INTRODUCTION

Dairy cattle production represents a key sector of modern agriculture, providing essential food products while simultaneously contributing to environmental challenges. Increasing demand for milk and dairy products requires improvements in production efficiency, but also emphasizes the need to reduce the environmental impact of livestock systems, particularly emissions of nitrogen compounds such as ammonia (FAO, 2009; Gerber et al., 2013; Dopelt et al., 2019).

Milk production and composition, including daily milk yield and milk components such as fat and protein, are influenced by a complex interaction of genetic, nutritional, and environmental factors. Differences in feeding strategies, herd management, and production systems can lead to considerable variability in production traits both within and between dairy production regions (Gantner and Barać, 2016; Gallardo and Teixeira, 2023). Understanding this variability is essential for improving productivity and optimizing management practices in dairy farming systems. In addition to production traits, nitrogen-related parameters such as milk urea and milk urea nitrogen (MUN) are important indicators of metabolic processes and feeding efficiency in dairy cows. These parameters reflect the balance between dietary protein and energy supply and provide insight into nitrogen utilization within

the animal (Eicher, 2004; Kohn et al., 2004). Milk urea nitrogen is closely related to urea concentration in blood and urine and can be used as a non-invasive indicator of nitrogen excretion and utilization (Roseler et al., 1993; Burgos et al., 2010; Spek et al., 2013).

Ammonia emissions represent a significant environmental concern in livestock production due to their impact on air quality and ecosystems. A substantial proportion of nitrogen excreted by dairy cows is converted into ammonia, contributing to environmental pollution (Hristov et al., 2011; Leip et al., 2015). Therefore, milk urea and MUN can be used not only as indicators of nutritional status but also as indirect indicators of potential ammonia emissions (Godden et al., 2001; Haig et al., 2002). Regional differences in dairy production systems, including variations in climate, feeding practices, and management conditions, may contribute to differences in production performance and nitrogen metabolism (Arsov, 1986; Gantner and Barać, 2016). Evaluating these regional variations is important for identifying opportunities to improve feeding strategies, enhance nitrogen utilization efficiency, and reduce environmental impacts.

Therefore, the aim of this study was to evaluate regional variability in daily milk yield, milk composition, milk urea, milk urea nitrogen, and ammonia emissions in dairy cows across different breeding regions of Croatia.

MATERIAL AND METHODS

The statistical analysis in this study was based on test-day records of Holstein cows collected over an 18-year period, from January 2005 to December 2022, as part of routine milk recording. The milk recording procedure followed the standards established by the International Committee for Animal Recording (ICAR, 2017) for alternative recording methods (AT4/BT4). This approach involved measuring milk yield and collecting milk samples from each cow during either the morning or evening milking at approximately four-week intervals. Data collection for the AT4 method was carried out by trained officers from the Croatian Agency for Agriculture and Food (HAPIH), while the BT4 method was performed by trained farm personnel.

Following collection, milk samples were transported to the accredited Central Laboratory for Milk Quality Control (SLKM) of HAPIH for analysis, ensuring compliance with ICAR (2017) protocols. Milk composition, including fat, protein, lactose, total solids, solids-not-fat, urea, casein, free fatty acids, pH, ketone bodies, and freezing point, was determined using infrared spectrophotometry (MilkoScan analyzers).

Prior to data validation, the dataset obtained from HAPIH comprised more than 5 million test-day records. Records that did not comply with ICAR (2017) standards, as well as those with incomplete or inconsistent information (breed, farm identification, breeding region, lactation stage, parity, age at first calving, calving date, and test-day date), were excluded. After data editing and quality control, the final dataset included 3,953,637 test-day records of Holstein cows. Farms were grouped into three breeding regions: Central, Eastern, and Mediterranean Croatia.

All statistical analyses were performed using SAS software (SAS Institute Inc., 2016), version 9.4.

RESULTS AND DISCUSSION

The results of the statistical analysis are presented by dairy cow breeding regions (Central, Eastern, and Mediterranean Croatia). Variability in production traits and nitrogen-related parameters is summarized in Tables 1 and 2.

Table 1. Basic statistical parameters of daily milk yield by dairy cow breeding region (Central Croatia, Eastern Croatia, and Mediterranean Croatia).

Trait	N	Mean	SD	CV, %	SE	Min	Max
Central Croatia							
DMY, kg	2237569	20.59	8.71	42.30	0.01	3.00	99.40
DFC, %	2277935	4.23	0.95	22.51	0.00	1.50	9.00
DPC, %	2288604	3.44	0.49	14.28	0.00	1.04	7.00
DFY, kg	2193854	0.86	0.39	45.79	0.00	0.05	7.94
DPY, kg	2203826	0.70	0.28	40.46	0.00	0.07	4.97
Eastern Croatia							
DMY, kg	2913627	24.39	10.22	41.92	0.01	3.00	99.90
DFC, %	2932266	4.15	1.00	23.98	0.00	1.50	9.00
DPC, %	2951872	3.46	0.46	13.30	0.00	1.02	7.00
DFY, kg	2843008	1.00	0.47	47.12	0.00	0.05	7.46
DPY, kg	2861521	0.83	0.32	39.94	0.00	0.06	4.44
Mediterranean Croatia							
DMY, kg	260023	23.12	9.36	40.50	0.01	3.00	98.90
DFC, %	255818	3.89	0.93	23.95	0.00	1.50	9.00
DPC, %	259056	3.39	0.46	13.64	0.00	1.62	6.98
DFY, kg	250424	0.88	0.38	43.27	0.00	0.05	5.71
DPY, kg	253623	0.77	0.29	37.60	0.00	0.06	4.85

*DMY – daily milk yield (kg); DFC – daily fat content (%); DPC – daily protein content (%); DFY – daily fat yield (kg); DPY - daily protein yield (kg); N - absolute number of cows; Mean - mean value; SD - standard deviation, CV - coefficient of variation; SE - standard error; Min - minimum, Max - maximum

The results presented in Table 1 indicate regional differences in dairy cow production traits, including daily milk yield (DMY), milk fat and protein content, and their respective daily yields across three regions of Croatia: Central, Eastern, and Mediterranean Croatia. These differences may reflect the combined effects of genetic background, environmental conditions, feeding practices, and management systems. In Central Croatia, the average daily milk yield was 20.59 kg, with high variability (SD = 8.71 kg; CV = 42.30%), indicating considerable differences among farms. Milk fat content averaged 4.23% (CV = 22.51%), while daily fat yield (0.86 kg) showed high variability (CV = 45.79%). Protein content was more stable (3.44%; CV = 14.28%), whereas daily protein yield (0.70 kg) exhibited substantial variability (CV = 40.46%). Eastern Croatia showed the highest average daily milk yield (24.39 kg), with similarly high variability (CV = 41.92%). Although milk fat content (4.15%) was slightly lower than in Central Croatia, daily fat yield was higher (1.00 kg), with the greatest variability (CV = 47.12%). Protein content (3.46%) was slightly higher and more stable (CV = 13.30%), while daily protein yield (0.83 kg) exceeded that of Central Croatia. In Mediterranean Croatia, average daily milk yield was 23.12 kg (CV = 40.50%), with variability comparable to the other regions. Milk fat content was the lowest (3.89%), while protein content (3.39%) remained relatively stable (CV = 13.64%). Daily protein yield (0.77 kg) showed somewhat lower variability (CV = 37.60%). Overall, Eastern Croatia achieved the highest production levels, while Central Croatia showed the greatest variability among farms. Mediterranean Croatia exhibited intermediate production levels but slightly lower milk component values. These regional differences may be associated with variation in feeding strategies, environmental conditions, and herd management practices. Central Croatia, characterized by high variability, may reflect more heterogeneous production systems, while Eastern Croatia appears to have more consistent management and feeding conditions. In Mediterranean Croatia, lower milk component values may be influenced by specific climatic conditions and seasonal feed availability. The observed patterns are consistent with findings reported in the literature. Gauly and Ammer

(2020) highlight the impact of climate change on dairy production systems in Europe, emphasizing that regions exposed to more extreme climatic conditions face greater challenges in maintaining stable milk production. Similarly, studies conducted in France and the Netherlands have demonstrated the influence of regional factors such as nutrition and feed quality on milk yield (Vanlinder et al., 2015), which aligns with the patterns observed in Croatia. Research from the USA and Australia has also shown that milk fat content is strongly influenced by feeding practices and diet composition (Moore et al., 2016; Nguyen et al., 2019). Furthermore, studies in Sweden and Canada report that greater stability in protein content is often associated with more uniform feeding practices and improved herd management (Smith et al., 2020), which corresponds to the lower variability observed in certain regions in this study. Overall, these findings highlight the importance of region-specific management and feeding strategies for optimizing milk production and composition.

In addition to production traits, variability in nitrogen-related parameters, including milk urea, milk urea nitrogen (MUN), and ammonia emissions, was also evaluated across the same dairy cow breeding regions. The results are presented in Table 2.

Table 2. Basic statistical parameters of milk urea, milk urea nitrogen (MUN), and ammonia emissions per cow across dairy cow breeding regions (Central Croatia, Eastern Croatia, and Mediterranean Croatia)

Trait	N	Mean	SD	CV, %	SE	Min	Max
Central Croatia							
UREA, mg/dL	2193192	20.04	10.09	50.36	0.01	0.50	60.00
MUN, mg/dL	2193192	9.22	4.64	50.36	0.00	0.23	27.60
AE, g/cow/day	2193192	71.37	23.35	32.72	0.02	26.16	163.83
Eastern Croatia							
UREA, mg/dL	2870767	23.28	8.90	38.25	0.01	0.50	60.00
MUN, mg/dL	2870767	10.71	4.10	38.25	0.00	0.23	27.60
AE, g/cow/day	2870767	78.87	20.60	26.12	0.01	26.16	163.83
Mediterranean Croatia							
UREA, mg/dL	252434	24.19	9.74	40.28	0.02	0.50	60.00
MUN, mg/dL	252434	11.13	4.48	40.28	0.01	0.23	27.60
AE, g/cow/day	252434	80.97	22.54	27.84	0.05	26.16	163.83

*UREA - Daily urea content in milk (mg/dL); MUN - Daily milk urea nitrogen content (mg/dL); EA - Daily ammonia emission (g/cow/day); N - total number of cows; Mean - mean value; SD - standard deviation; CV - coefficient of variation; SE - standard error; Min - minimum; Max - maximum

The results presented in Table 2 show considerable variability in daily milk urea content (UREA), milk urea nitrogen (MUN), and ammonia emissions per cow (AE) across dairy cow breeding regions (Central Croatia, Eastern Croatia, and Mediterranean Croatia). These differences may reflect the influence of geographical and climatic conditions, as well as variation in feeding practices, production intensity, and herd management. Daily milk urea content (UREA) differed among regions, with the highest average value recorded in Mediterranean Croatia (24.19 mg/dL) and the lowest in Central Croatia (20.04 mg/dL). Eastern Croatia showed intermediate values (23.28 mg/dL). The coefficient of variation was highest in Central Croatia (50.36%), indicating substantial variability among cows and farms, while the lowest variability was observed in Eastern Croatia (38.25%), suggesting more consistent feeding and management conditions. Milk urea nitrogen (MUN) followed a similar pattern, with the highest average values in Mediterranean Croatia (11.13 mg/dL) and the lowest in Central Croatia (9.22 mg/dL). Eastern Croatia again showed intermediate values (10.71 mg/dL). Variability in MUN was highest in Central Croatia (50.36%) and lowest in Eastern Croatia (38.25%), indicating differences in production uniformity among regions. Ammonia emissions per cow (AE) also varied among regions, with the highest values recorded in Mediterranean Croatia (80.97 g/cow/day) and the lowest in Central Croatia (71.37 g/cow/day). Eastern

Croatia showed relatively high emissions (78.87 g/cow/day). Variability was greatest in Central Croatia (CV = 32.72%) and lowest in Eastern Croatia (CV = 26.12%). The observed regional patterns suggest that higher urea and MUN concentrations, as well as increased ammonia emissions in Mediterranean Croatia, may be associated with higher dietary protein levels and differences in feed availability under specific climatic conditions. Eastern Croatia, characterized by more intensive production systems, showed relatively high but more consistent values, indicating more uniform feeding strategies and herd management. In contrast, Central Croatia exhibited lower average values but higher variability, which may reflect greater heterogeneity in feeding practices, housing conditions, and overall management. Overall, these findings highlight the importance of regional factors in shaping nitrogen metabolism and ammonia emissions in dairy production systems. The results suggest that adaptation of feeding strategies and management practices to specific regional conditions may improve nitrogen utilization efficiency and contribute to the reduction of environmental impacts.

CONCLUSION

The results of this study demonstrate clear regional variability in milk production traits, milk urea, milk urea nitrogen (MUN), and ammonia emissions in dairy cows in Croatia. Eastern Croatia achieved the highest production levels, while Central Croatia showed lower average values but greater variability among farms. Mediterranean Croatia exhibited intermediate production levels, but the highest values of urea, MUN, and ammonia emissions. The observed differences among regions may be associated with variations in feeding strategies, production intensity, and management practices. Higher values of urea and MUN, together with increased ammonia emissions, suggest less efficient nitrogen utilization and a greater potential environmental impact, particularly in Mediterranean and Eastern Croatia. In contrast, lower values observed in Central Croatia may reflect more balanced feeding conditions, although the high variability indicates the need for improved consistency in management. Overall, the findings highlight the importance of region-specific approaches in dairy production. Optimization of feeding strategies and herd management adapted to regional conditions may contribute to improved production efficiency and reduced nitrogen losses and ammonia emissions.

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REFERENCES

- Arsov, A., Golc, S., & Kastelic, D. (1986). Higijensko pridobivanje mleka. Knjižica za pospeševanje kmetijstva. Ljubljana, ČZP Kmečki glas.
- Burgos, S.A., Embertson, N.M., Zhao, Y., Mitloehner, F.M., DePeters, E.J. & Fadel, J.G. (2010). Prediction of ammonia emission from dairy cattle manure based on milk urea nitrogen: Relation of milk urea nitrogen to ammonia emissions. *Journal of Dairy Science*, 93(6), 2377-2386. <https://doi.org/10.3168/jds.2007-0299>
- Dopelt, K., Radon, P., & Davidovitch, N. (2019). Environmental Effects of the Livestock Industry: The Relationship between Knowledge, Attitudes, and Behavior among Students in Israel. *International Journal of Environmental Research and Public Health*, 16(8), 1359. <https://doi.org/10.3390/ijerph16081359>
- Eicher, R. (2004). Evaluation of the metabolic and nutritional situation in dairy herds: Diagnostic use of milk components. 23rd World Buiatrics Congress, Quebec City, Canada.
- FAO (2009). The State of Food and Agriculture - Livestock in the Balance. Food and Agriculture Organization, Rome, Italy.

- Gallardo, W.B., & Teixeira, I. (2023). Associations between Dietary Fatty Acid Profile and Milk Fat Production and Fatty Acid Composition in Dairy Cows: A Meta-Analysis. *Animals*, 13(13), 2063. <https://doi.org/10.3390/ani13132063>
- Gantner, V., & Barać, Z. (2016). Uzgojno-seleksijski rad u stočarstvu. Poljoprivredni fakultet u Osijeku. Sveučilište Josipa Jurja Strossmayera u Osijeku.
- Gauly, M., & Ammer, S. (2020). Review: Challenges for dairy cow production systems arising from climate changes. *Animal*, 14(1), 196-203. <https://doi.org/10.1017/S1751731119003239>
- Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., & Tempio, G. (2013). Tackling climate change through livestock: A global assessment of emissions and mitigation opportunities. Rome: Food and Agriculture Organization of the United Nations (FAO).
- Godden, S.M., Lissemore, K.D., Kelton, D.F., Leslie, K.E., Walton, J.S., & Lumsden, J.H. (2001). Relationships between milk urea concentrations and nutritional management, production and economic variables in Ontario dairy cows. *Journal of Dairy Science*, 84, 1128-1139. [https://doi.org/10.3168/jds.S0022-0302\(01\)74573-0](https://doi.org/10.3168/jds.S0022-0302(01)74573-0)
- Haig, P.A., Mutsvangwa, T., Spratt, R., & McBride, B.W. (2002). Effects of dietary protein solubility on nitrogen losses from lactating dairy cows and comparison with predictions from the Cornell net carbohydrate and protein system. *Journal of Dairy Science*, 85, 1208-1217. [https://doi.org/10.3168/jds.S0022-0302\(02\)74184-2](https://doi.org/10.3168/jds.S0022-0302(02)74184-2)
- Hristov, A.N., Hanigan, M., Cole, A., Todd, R., McAllister T.A., Ndegwa, P., & Rotz, A. (2011). Review: Ammonia emissions from dairy farms and beef feedlots. *Canadian Journal of Animal Science*, 91(1): 1-35. <https://doi.org/10.4141/CJAS10034>
- ICAR – International Committee for Animal Recording (2017). Section 2 - Guidelines for Dairy Cattle Milk Recording.
- Kohn, R.A., French, K.R., & Russek-Cohen, E. (2004). A Comparison of Instruments and Laboratories Used to Measure Milk Urea Nitrogen in Bulk-Tank Milk Samples. *Journal of Dairy Science*, 87, 1848-1853. [https://doi.org/10.3168/jds.S0022-0302\(04\)73342-1](https://doi.org/10.3168/jds.S0022-0302(04)73342-1)
- Leip, A., Billen, G., Garnier, J., Grizzetti, B., Lassaletta, L., Reis, S., Simpson, D., Sutton, M.A., de Vries, W., Weiss, F., & Westhoek, H. (2015). Impacts of European livestock production: nitrogen, sulphur, phosphorus and greenhouse gas emissions, land-use, water eutrophication and biodiversity. *Environmental Research Letters*, 10, 115004. <https://doi.org/10.1088/1748-9326/10/11/115004>
- Moore, S.A.E., & Kalscheur, K.F. (2016). Canola meal in dairy cow diets during early lactation increases production compared with soybean meal. *Journal of Animal Science*, 94, 731. <https://doi.org/10.2527/jam2016-1506>
- Nguyen, M., Pham, D., Hostiou, N., Cournut, S., Guillaume, D., Culas, C., & Pannier, E. (2019). Dairy farming, a clash of production models. In CesaroJD, Duteurtre G and Nguyen MH (eds), Atlas of Livestock Transitions in Vietnam: 1986–2016. Hanoi: IPSARD-CIRAD, pp. 47-56.
- Roseler, D.K., Ferguson, J.D., Sniffen, C.J., & Herrema, J. (1993). Dietary protein degradability effects on plasma and milk urea nitrogen and milk non-protein nitrogen in Holstein cows. *Journal of Dairy Science*, 76, 525. [https://doi.org/10.3168/jds.S0022-0302\(93\)77372-5](https://doi.org/10.3168/jds.S0022-0302(93)77372-5)
- SAS Institute Inc. (2016). SAS® 9.4 User's Guide. Cary, NC, USA: SAS Institute Inc.
- Smith, G.N., Brok, E., Vormsborg Christiansen, M., & Ahrné, L. (2020). Casein micelles in milk as sticky spheres. *Soft Matter*, 16, 9955-9963. <https://doi.org/10.1039/D0SM01327G>
- Spek, J.W., Dijkstra, J., Duinkerken, G.V., Bannik, A. (2013). A review of factors influencing milk urea concentration and its relationship with urea excretion in lactating dairy cattle. *Journal of Agricultural Science*, 151, 407-423. <https://doi.org/10.1017/S0021859612000561>
- Vanlierde, A., Vanrobays, M.L., Dehareng, F., Froidmont, E., Soyeurt, H., McParland, S., Lewis, E., Deighton, M.H., Grandl, F., Kreuzer, M., Gredler, B., Dardenne, P., & Gengler, N. (2015). Hot topic: Innovative lactation-stage-dependent prediction of methane emissions from milk mid-infrared spectra. *Journal of Dairy Science*, 98(8), 5740-5747. <https://doi.org/10.3168/jds.2014-8436>

Effects of whey and highly digestible protein sources in starter feeds on growth performance and morphometric development of early-weaned Holstein Friesian calves

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Abstract

The aim of the present study was to evaluate the effects of incorporating whey and easily digestible protein sources into pelleted starter feeds on growth performance and overall development of calves. Forty Holstein Friesian calves were assigned to four feeding groups, receiving either a standard starter mix or experimental diets supplemented with whey, yeast nucleotides, soybean protein concentrate, and limiting amino acids. Body weight and detailed morphometric measurements were recorded at multiple time points from birth to three months of age, and growth trajectories were analyzed using LSmeans and correlation analyses. The inclusion of whey and digestible protein sources did not significantly alter growth parameters at weaning, but female calves from experimental groups demonstrated significantly greater withers height (WH) and differences in skeletal development by three months of age. These results indicate that dietary supplementation with whey and digestible proteins were associated with differences in structural growth beyond the weaning period without adversely affecting early postnatal growth, supporting the strategic use of such ingredients in calf starter feeds to optimize developmental outcomes.

Keywords: Holstein Friesian calves, whey, easily digestible proteins, starter mix, body traits

INTRODUCTION

Monitoring the growth and development of calves represents a fundamental component of efficient and sustainable cattle production, particularly during the early postnatal period characterized by intensive physiological, metabolic, and structural changes. The first two months of life are considered especially critical, as growth patterns established during this phase have long-lasting implications for animal performance. Increasing scientific evidence indicates that early-life nutrition and management practices influence future productivity through mechanisms of metabolic programming and epigenetic regulation (Khan et al., 2011; Soberon et al., 2012; Brickell et al., 2009). These processes affect not only growth efficiency but also milk production potential, health status, and overall longevity of dairy cattle. Growth performance during the pre-weaning period is closely associated with key productive and reproductive parameters later in life, including age at first insemination, disease resistance, and lifetime milk yield. In this context, birth weight represents a fundamental baseline parameter for assessing early development and serves as a reference point for calculating average daily gain (ADG), one of the most widely used indicators of growth efficiency. Achieving optimal growth rates during this stage reflects adequate nutritional management and is essential for ensuring proper physiological development and welfare of calves.

Furthermore, considerable variability in growth performance has been observed under commercial farming conditions, primarily due to differences in feeding regimes, housing systems, environmental conditions, and health management. Johnson et al. (2018) evaluated these factors across 11 commercial dairy farms, including 492 calves, predominantly of the Holstein-Friesian breed. Morphometric measurements, including body weight, height, and body length, were recorded at weeks 1, 5, and 9 of age to assess growth dynamics. The study reported that calves were mainly fed milk replacer, although variations in feeding strategies were present, including the use of acidified whole milk. Daily liquid feed intake ranged from 4 to 6 liters, with milk replacer concentrations between 10% and 15%. The results demonstrated that growth intensity increased during the second month of life compared to the first, with average daily gain ranging from 0.48 to 0.71 kg/day in the early pre-weaning phase and reaching approximately 0.83 ± 0.16 kg/day after weaning. Similar trends were observed for skeletal growth, including increases in body height and length. However, substantial variability in growth performance among calves within the same farm was also reported, which was attributed to differences in feeding intensity, ambient temperature, and health status. In particular, restricted liquid feeding was identified as a key limiting factor for achieving optimal growth, highlighting the importance of adequate nutritional supply during this critical developmental stage.

Despite the growing body of knowledge on early-life nutrition, there is still limited information regarding the effects of specific dietary components, such as whey and highly digestible protein sources, when included in starter feeds under practical production conditions. These components may play a significant role in improving nutrient availability, supporting rumen development, and enhancing overall growth performance in calves.

Therefore, the aim of the present study was to evaluate the effects of incorporating whey and easily digestible protein sources into pelleted starter feeds on growth performance and overall development of calves. It was hypothesized that such dietary modifications would positively influence growth dynamics and contribute to improved developmental outcomes during the pre-weaning period.

MATERIAL AND METHODS

The experimental trial was conducted on two commercial livestock farms, Mitrovac and Karanac, managed by Belje plus d.o.o., involving a total of 40 Holstein Friesian calves (20 males and 20 females). Calves were randomly assigned to four feeding groups, with each group consisting of 10 animals (5 males and 5 females), balanced according to birth weight. Housing, feeding regime, and general management conditions were consistent across all experimental groups within each farm and followed standard management practices of Belje plus d.o.o. The experimental design included both sexes; however, the duration of the experimental period differed between male and female calves due to production-related management practices. Male calves were monitored until slaughter at an average age of 56 days, while female calves were followed until 91 days of age to enable evaluation of post-weaning growth. Female calves from all groups were transferred from the Mitrovac farm, a dairy production facility, to the Karanac farm, dedicated to calf and heifer rearing, at an average age of 63 days. This transfer represents a standard management practice within the production system of Belje plus d.o.o. and was applied uniformly across all experimental groups. A detailed overview of the experimental design is provided in Table 1.

Table 1. Research plan

Group	Number of animals	Treatment
C	10	Milk replacer + GT-1 18% SB, control
E1	10	Milk replacer + GT-1 18% SB with whey
E2	10	Milk replacer + GT-1 18% SB with nucleotides, soybean protein concentrate, and amino acids
E3	10	Milk replacer + GT-1 18% SB with whey, nucleotides, soybean protein concentrate, and amino acids

*C – Control group, E1 – First experimental group, E2 – Second experimental group, E3 – Third experimental group

During the study, all calves were fed a commercial milk replacer (MR) using a "step-down" feeding protocol. Specifically, from day 1 to day 35, calves received two daily meals of 3 L MR (totaling 6 L/day); from day 36 to day 42, two daily meals of 2 L MR (totaling 4 L/day) were administered; and from day 43 to day 49, calves were offered one daily meal of 2 L MR. The MR was prepared at a concentration of 143 g/L of water, or equivalently 125 g/L of solution. The formulation of the MR included high-quality protein sources, consisting of whey proteins with an estimated digestibility of 98% and hydrolyzed wheat proteins with a digestibility of approximately 92%, as well as a lipid fraction composed of 40% coconut oil and 60% palm oil. This combination of oils was selected to approximate the fatty acid profile of natural milk fat. The fat fraction was homogenized into globules smaller than 3 µm, resulting in an estimated digestibility of 87%. Proteins were highlighted as the costliest component of the MR, with considerable variation in digestibility, amino acid composition, and the presence of anti-nutritional factors. Pelleted starter feed (GT-1 18% SB) was offered ad libitum from day 4 of life, alongside ad libitum access to water. Hay was not included in the diet until weaning. The starter feed was produced using a Bühler pellet mill with a 4.5 × 60/50 matrix, achieving a Pellet Durability Index (PDI) of 95 across all four formulations used in the trial. The pelleted form was chosen to enhance voluntary feed intake and minimize feed wastage. Although all four starter feed formulations maintained consistent nutritional value, they differed in raw material composition to reflect the experimental treatments for the four feeding groups.

Experimental diets and growth monitoring

Calves were assigned to four dietary treatment groups. The control group received a standard starter mix routinely used on Belje plus d.o.o. farms. Experimental group E1 was fed a starter mix in which a portion of the corn content was replaced with dry sweet whey, serving as a lactose source. Experimental group E2 received a starter mix in which soybean meal and rapeseed meal, as primary protein sources, were partially substituted with alternative protein ingredients, including yeast nucleotides, soybean protein concentrate, and two limiting amino acids—methionine and lysine. Experimental group E3 was provided with a starter mix combining the modifications of E1 and E2, containing dry whey, yeast nucleotides, soybean protein concentrate, and the limiting amino acids methionine and lysine. All calves were weaned at an average age of 50 days. Male calves continued to receive their respective starter mix until slaughter at an average age of 56 days, while female calves were fed the same starter mix until an average age of 63 days, after which they were transferred to the Karanac farm and transitioned to a total mixed ration (TMR).

Body weight measurements and average daily gain

Birth weight was recorded individually for each calf immediately after parturition using a calibrated livestock scale. Subsequent body weight measurements were conducted at approximately 6, 24, 50, and 91 days of age (the latter exclusively for female calves). For male calves, the final body weight measurement was obtained at 56 days of age, corresponding to the day of slaughter. These weight measurements were used to calculate average daily gain (ADG) for each feeding group, separated by sex, and for specific periods, as well as for the overall duration of the study.

Calf growth was further assessed through detailed morphometric measurements taken at four time points during the study: 6, 24, 50, and 91 days (the latter exclusively for female calves). The following parameters were measured using a combination of a Lydtin stick and measuring tape:

- withers height: vertical measurement from the floor to the top of the withers along the front leg.
- croup height: vertical measurement from the floor to the anterior edge of the sacral bone where the midline of the spine intersects the line connecting the tuber coxae.
- body length: distance from the center of the scapulohumeral joint to the ischial tuberosity (*tuber ischiadicum*).
- pelvic width: lateral distance between the outer edges of the tuber coxae.
- chest depth: vertical distance from the withers to the sternum.
- chest width: measured immediately posterior to the shoulder blades.
- chest girth: circumference measured over the highest point of the withers and across the center of the sternum.
- body girth: circumference at the level of the last rib.

- metacarpus circumference: circumference at the midpoint of the metacarpus of the foreleg.
- buttock length: linear distance from the heel (tuber calcanei) to the ischial tuberosity.

These morphometric parameters, combined with body weight data, were used to assess the overall growth, development, and body conformation of calves under different dietary treatments.

Statistical analysis

All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA), version 9.4. Data were analyzed using mixed models, with dietary treatment, sex, and their interaction as fixed effects, and individual animal as a random effect. Least squares means (LSMeans) were calculated to compare treatment effects. In addition, correlation analyses were performed to examine relationships between morphometric measurements, body weight, and average daily gain. Statistical significance was inferred at $p < 0.05$. Due to differences in experimental duration between sexes, analyses involving later growth stages (91 days) were conducted exclusively on female calves, while earlier growth stages were evaluated using the full dataset.

RESULTS AND DISCUSSION

Production performance was evaluated through longitudinal body weight measurements complemented by detailed morphometric traits. The relationships among measured variables are presented in Table 2, which summarizes Pearson correlation coefficients across all observations.

Table 2. Pearson correlation coefficients between body measurements of calves (n = 140).

Trait	BM	WH	CH	BL	CD	CW	CG	BG	MC	CWd	TL	LG
BM	1	0.902	0.908	0.920	0.921	0.881	0.964	0.977	0.553	0.908	0.883	0.760
WH		1	0.912	0.873	0.891	0.819	0.905	0.883	0.507	0.851	0.845	0.646
CH			1	0.870	0.901	0.797	0.919	0.878	0.519	0.854	0.874	0.693
BL				1	0.899	0.828	0.906	0.900	0.550	0.867	0.833	0.669
CD					1	0.827	0.933	0.915	0.524	0.839	0.829	0.686
CW						1	0.871	0.852	0.524	0.830	0.780	0.720
CG							1	0.958	0.554	0.896	0.913	0.762
BG								1	0.498	0.877	0.856	0.740
MC									1	0.535	0.520	0.470
CWd										1	0.847	0.696
TL											1	0.699

*BM – body weight; WH – withers height; CH – croup height; BL – body length; CD – chest depth; CW – chest width; CG – chest girth; BG – body girth; MC – metacarpus circumference; CWd – croup width; TL – thigh length; LG – lifetime growth. n represents the total number of repeated observations across measurement time points, including measurements at 6, 24, and 50 days for all calves and measurements at 91 days for female calves only.

A consistently high degree of positive association was observed among most morphometric traits and body weight, with coefficients exceeding 0.90 for key structural parameters such as body length, chest girth, and body girth. The strongest relationships were identified between body mass and body girth ($r = 0.977$) and chest girth ($r = 0.964$), confirming the reliability of thoracic and abdominal measurements as predictors of live weight. These findings agree with Heinrichs et al. (1992) and Davis (1954), who emphasized chest girth as the most reliable estimator of body mass in growing cattle. Compared to Sahu et al. (2017), higher correlation coefficients were observed in the present study, which may reflect reduced environmental variability and a more uniform genetic background. Moderate correlations involving metacarpus circumference suggest that peripheral skeletal development is less tightly coupled with overall body mass, whereas the positive association with lifetime growth indicates its relevance as an auxiliary structural indicator. Furthermore, estimated least squares means of body measurements according to feeding treatment are presented in Table 3.

Table 3. LSmeans of measurements of body traits according to the feeding group.

Body trait	C	E1	E2	E3
Birth weight (kg)	41.02	42.23	40.31	41.29
Body weight (kg)	58.82 ^{ab}	61.29 ^a	57.74 ^b	59.88 ^{ab}
Withers height (cm)	80.69 ^a	82.47 ^b	81.32 ^{ab}	81.73 ^{ab}
Croup height (cm)	84.90	85.82	84.54	85.66
Body length (cm)	76.58	77.36	75.67	76.09
Chest depth (cm)	31.72	32.31	31.63	32.19
Chest width (cm)	15.51	15.95	16.09	15.81
Chest girth (cm)	89.15	90.07	88.61	89.70
Body girth (cm)	97.45 ^a	100.17 ^b	97.54 ^a	99.14 ^{ab}
Metacarpus circumference (cm)	11.12	11.33	11.22	11.06
Croup width (cm)	23.77	23.69	23.45	23.53
Thigh length (cm)	43.36 ^a	42.32 ^b	42.19 ^b	42.80 ^{ab}

*Different superscripts within rows indicate differences between groups ($p < 0.05$).

Calves receiving diets containing whey (E1 and E3) showed higher values for body weight and selected morphometric traits compared to the control and E2 groups. The highest body weight was observed in E1, while E2 showed lower values. Statistically significant differences ($p < 0.05$) between feeding groups were observed for body weight, withers height, body girth, and thigh length, as presented in Table 3. Similar tendencies have been reported by Khan et al. (2007b) and DePeters et al. (1986), who observed improved growth under diets with higher levels of readily fermentable energy sources. Temporal development of body measurements and growth rates is summarized in Table 4.

The LSmeans presented in Table 4 provide a comprehensive overview of growth dynamics and body conformation of Holstein Friesian calves subjected to four different dietary treatments. At the first measurement (6 days), statistically significant differences ($P < 0.05$) were observed in withers height (WH) and body girth, with E1 calves showing the highest values compared to the other groups, as presented in Table 4. These results indicate differences in early skeletal development between feeding groups, consistent with Khan et al. (2011) and Brickell et al. (2009), who reported the importance of early-life nutrition for skeletal growth. At the second measurement (24 days), significant differences emerged in croup width (CWd) and thigh length (TL). Control calves had a larger CWd (23.37 cm) and TL (43.32 cm) than E3 and E2 calves (CWd 21.82–22.87 cm; TL 40.78–41.72 cm), indicating variability in hindquarter development possibly related to protein source composition, in line with findings of DePeters et al. (1986) and Soberon et al. (2012).

At the second measurement (24 days), no statistically significant differences were observed for most morphometric traits, although numerical differences between feeding groups were present. These results corroborate Franklin et al. (1998) and Heinrichs & Hargrove (1987), who reported similar withers height values (≈ 80 cm) at 4 weeks. By the third measurement (50 days), no statistically significant differences ($P > 0.05$) were found among groups for most body traits, although E1 calves exhibited the highest BW (64.67 kg) and ADG (0.454 kg/day), while E2 had the lowest BW (61.48 kg, ADG 0.439 kg/day). These trends indicate differences in growth performance between feeding groups, aligning with DePeters et al. (1986) and Khan et al. (2007b), who reported growth rates around 0.5 kg/day for similar rearing conditions.

At the final measurement (91 days, female calves), WH and BL differed significantly among groups ($P < 0.05$), with E1 calves reaching the highest WH (92.25 cm) and BL (90.40 cm), while E2 calves showed the lowest BL (83.51 cm). Lifetime growth (LG) also reflected cumulative dietary effects, with E1 and E3 achieving superior LG (0.626–0.663 kg/day) compared to E2 (0.540 kg/day). These results indicate differences in skeletal development between feeding groups at later stages, corroborating the observations of Franklin et al. (1998) and Saegusa et al. (2017). Furthermore, the integration of whey and high-digestibility protein sources (E1 and E3) enhanced both early and later growth parameters, with body girth,

withers height, and body length serving as reliable indicators of nutritional efficacy. The observed correlations between morphometric traits and body weight further support the utility of these measures in predicting growth trajectories (Heinrichs et al., 1992; Sahu et al., 2017; Davis, 1954).

Table 4. LSmeans of body trait measurements of calves at different ages according to feeding group (n = 40; female calves measured at 91 days, n = 20).

Trait / age	Feeding groups			
	C	E1	E2	E3
Body weight, kg				
6th day	42.52	43.55	41.70	42.52
24th day	50.11	53.33	49.70	50.41
50th day	62.87	64.67	61.48	63.83
91st day	93.33	97.19	87.26	99.32
Withers height, cm				
6th day	77.17 ^A	80.03 ^B	79.11 ^{ABC}	77.79 ^{AC}
24th day	80.03	81.34	80.14	80.14
50th day	84.37	83.58	83.24	84.91
91st day	86.70 ^A	92.25 ^B	90.90 ^{AB}	90.94 ^B
Croup height, cm				
6. day	80.43	81.03	79.81	80.04
24. day	82.32	85.05	82.68	84.20
50. day	88.63	87.03	88.91	89.53
91. day	94.82	97.72	93.68	96.59
Body length, cm				
6th day	67.82	71.31	69.67	69.15
24th day	72.08	75.25	74.20	73.02
50th day	80.50	76.93	76.92	78.20
91st day	90.42 ^{AB}	90.40 ^B	83.51 ^A	90.07 ^{AB}
Chest depth, cm				
6th day	28.23	29.17	28.71	27.57
24th day	29.05	31.43	29.50	31.12
50th day	33.75	32.48	33.30	33.52
91st day	39.54	38.97	38.58	39.80
Chest width, cm				
6th day	13.71	13.47	14.34	13.85
24th day	15.16	15.82	16.13	15.50
50th day	16.42	16.85	16.40	16.38
91st day	19.37	20.48	20.03	19.42
Chest girth, cm				
6th day	80.88	81.42	80.07	80.17
24th day	85.68	87.50	86.24	86.73
50th day	94.43	95.47	93.39	95.76
91st day	108.87	108.73	107.92	109.28
Body girth, cm				
6th day	81.98 ^{AB}	85.50 ^A	80.79 ^B	83.13 ^{AB}
24th day	89.11	91.89	89.75	89.80
50th day	98.50	100.53	98.83	100.04
91st day	128.36	128.99	125.45	133.21

*Different superscripts within rows indicate differences between groups (p < 0.05).

Finally, the obtained results indicate that dietary modifications, particularly the inclusion of whey, may influence growth performance and structural development in calves. The strong interrelationships among morphometric traits further confirm their utility as reliable indicators of growth dynamics and provide a robust framework for evaluating nutritional interventions in early-life cattle production systems.

CONCLUSION

The study analysed the effects of incorporating whey and highly digestible protein sources into starter mixes on the growth and body development of early-weaned Holstein Friesian calves. The results indicated that the inclusion of whey and digestible protein sources did not produce statistically significant differences in body weight, withers height, croup height, body length, chest depth, chest width, chest girth, body girth, metacarpus circumference, croup width, thigh length, or overall lifetime growth at weaning compared to calves receiving the standard control diet. This suggested that early postnatal growth was not markedly influenced by variations in the protein composition of starter feeds under the conditions studied. By three months of age, female calves from the experimental groups receiving whey and digestible protein sources exhibited significantly greater withers height compared to the control group. Body length was also significantly greater in these groups, while overall lifetime growth showed higher numerical values. These results indicate differences in skeletal development between feeding groups during later stages. The obtained results indicate that although immediate post-weaning traits were largely unaffected, early-life feeding strategies incorporating whey and digestible proteins were associated with improved structural growth in female calves and differences in growth trajectories beyond weaning.

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REFERENCES

- Brickell, J. S., Bourne, N., McGowan, M. M., & Wathes, D. C. (2009). Effect of growth and development during the rearing period on the subsequent fertility of nulliparous Holstein-Friesian heifers. *Theriogenology*, 72, 408–416. <http://doi.org/10.1016/j.theriogenology.2009.03.015>
- Davis, H. P. (1954). Relationship between weight and seven other body measurements of Holstein females at birth, 6 months, 12 months, 18 months and 24 months. *Journal of Animal Science*, 13, 1018.
- De Peters, E. J., Fisher, L. J., & Stone, J. L. (1986). Effect of adding dried whey to starter diet of early and late weaned calves. *Journal of Dairy Science*, 69, 181–186. [https://doi.org/10.3168/jds.S0022-0302\(86\)80384-8](https://doi.org/10.3168/jds.S0022-0302(86)80384-8)
- Franklin, S. T., Sorenson, C. E., & Hammel, D. C. (1998). Influence of vitamin A supplementation in milk on growth, health, concentrations of vitamins in plasma, and immune parameters of calves. *Journal of Dairy Science*, 81, 2623–2632. [https://doi.org/10.3168/jds.S0022-0302\(98\)75820-5](https://doi.org/10.3168/jds.S0022-0302(98)75820-5)
- Heinrichs, A. J., & Hargrove, G. L. (1987). Standards of weight and height for Holstein heifers. *Journal of Dairy Science*, 70, 653–660. [https://doi.org/10.3168/jds.S0022-0302\(87\)80055-3](https://doi.org/10.3168/jds.S0022-0302(87)80055-3)
- Heinrichs, A. J., Rogers, G. W., & Cooper, J. B. (1992). Predicting body weight and wither height in Holstein heifers using body measurements. *Journal of Dairy Science*, 75, 3576–3581. [https://doi.org/10.3168/jds.S0022-0302\(92\)78134-X](https://doi.org/10.3168/jds.S0022-0302(92)78134-X)
- Johnson, K. F., Chancellor, N., Burn, C. C., & Wathes, D. C. (2018). Analysis of preweaning feeding policies and other risk factors influencing growth rates in calves on 11 commercial dairy farms. *Animal*, 12(7), 1413–1423. <https://doi.org/10.1017/S1751731117003160>

- Khan, M. A., Lee, H. J., Lee, W. S., Kim, H. S., Kim, S. B., Ki, K. S., Park, S. J., Ha, J. K., & Choi, Y. J. (2007b). Starch source evaluation in calf starter: I. Feed consumption, body weight gain, structural growth, and blood metabolites in Holstein calves. *Journal of Dairy Science*, 90, 5259–5268. <https://doi.org/10.3168/jds.2007-0338>
- Khan, M. A., Weary, D. M., & von Keyserlingk, M. A. G. (2011). Invited review: Effects of milk ration on solid feed intake, weaning, and performance in dairy heifers. *Journal of Dairy Science*, 94, 1071–1081. <https://doi.org/10.3168/jds.2010-3733>
- Saegusa, A., Inouchi, K., Ueno, M., Inabu, Y., Koike, S., Sugino, T., & Oba, M. (2017). Effects of partial replacement of corn grain with lactose in calf starters on ruminal fermentation and growth performance. *Journal of Dairy Science*, 100, 6177–6186. <https://doi.org/10.3168/jds.2017-12616>
- Sahu, S. S., Choursia, S. K., Chaturvedani, A. K., & Prakash, O. (2017). Correlation between body weight and linear body measurements in adult female Sahiwal cattle. *Indian Journal of Veterinary Sciences & Biotechnology*, 12(3), 90–93. <http://dx.doi.org/10.21887/ijvsbt.v12i3.7103>
- Soberon, F., Raffrenato, E., Everett, R. W., & van Amburgh, M. E. (2012). Prewaning milk replacer intake and effects on long-term productivity of dairy calves. *Journal of Dairy Science*, 95, 783–793. <https://doi.org/10.3168/jds.2011-4391>

Physicochemical evaluation of traditional Janj cheese with selected plant additives

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Abstract

Janj cheese is a traditional dairy product from the Janj region in Bosnia and Herzegovina, produced from raw milk using specific artisanal technology. This study aimed to evaluate the effects of selected plant additives on the physicochemical properties of traditional Janj cheese. Twelve cheese samples were produced in two batches. Each batch included one control sample and five samples supplemented with oregano, basil, parsley, rosemary, and chives as plant additives. In the first batch, the additives were applied at a concentration of 0.50%, and in the second batch at 1.00%. Physicochemical analyses included moisture, dry matter, fat, protein, ash, sodium chloride content, pH, titratable acidity, water activity, instrumental color (CIE Lab*), and texture parameters. Moisture content ranged from 53.39% to 57.72% in the first batch and from 48.96% to 51.31% in the second batch. Fat content in dry matter ranged from 38.62% to 45.18%, while protein content varied between 18.12% and 25.05%, confirming the high nutritional value of Janj cheese. Ash and sodium chloride contents varied depending on the type of plant additive used. Differences in pH and titratable acidity reflected variations in fermentation intensity among samples. Water activity ranged from 0.901 to 0.932, while instrumental color measurements showed significant differences within each batch. The results indicate that the addition of plant additives significantly influenced ($p < 0.05$) the physicochemical properties of Janj cheese. Traditional production methods combined with local technological variations result in cheeses with distinct characteristics. Physicochemical evaluation plays a key role in maintaining the quality, safety, and distinctive character of traditional cheeses such as Janj, reflecting their cultural and economic importance. These findings highlight the potential of plant additives to modulate cheese properties while preserving the authentic features of this regional product.

Keywords: Janj cheese, plant additives, physicochemical properties

INTRODUCTION

Cheese is a complex food product that combines traditional knowledge and scientific principles, where specific milk processing techniques yield a product rich in nutritional value and characterized by distinctive sensory properties (Popović-Vranješ, 2015). Beyond its role as a staple food, cheese has gained increasing gastronomic importance, especially in countries with high living standards, where its consumption continues to rise. Traditional cheeses produced by indigenous methods are particularly valued for reflecting the culture, heritage, and unique environmental conditions of their regions (Dozet & Maćej, 2006; Keskin & Dağ, 2020; Cardin et al., 2022). Traditional cheeses not only provide essential nutrients and exhibit complex microbiological and sensory characteristics (Montel et al., 2014), but they also contribute to the preservation of cultural identity and gastronomic heritage, as culturally significant local food products play a central role in shaping place identity and heritage-based experiences (Vujko et al., 2025). Typically made from the milk

of local animal breeds using artisanal methods passed down through generations, traditional cheeses maintain distinctive sensory attributes and microbial diversity essential for fermentation and ripening, and are often named after their geographic origin (Popović-Vranješ, 2015).

Indigenous cheeses have long contributed to the diet and economy of rural populations. However, industrialization has threatened their continuity, prompting the need for preservation efforts. In Bosnia and Herzegovina, research has supported the protection and promotion of traditional dairy products and indigenous production technologies (Dozet, 2004; Bijeljac & Sarić, 2005; Dozet & Maćej, 2006). Production of such cheeses is predominantly household-based, with limited industrial representation (Bijeljac & Sarić, 2005). These cheeses are typically made from raw, unpasteurized milk, sometimes with natural starters, and involve significant manual labor. Their taste, aroma, texture, and microbial composition are more complex than those of industrial cheeses, offering richer sensory profiles (Kalit, 2015).

Janj cheese, the subject of this study, is a traditional braided cheese from the Janj region (Šipovo municipality, Republika Srpska, Bosnia and Herzegovina), produced from unpasteurized cow's milk. Its production is maintained within small rural households, where knowledge is traditionally inherited, often by younger women. The pristine environment of Mount Vitorog contributes to the high quality of the milk and the final product, emphasizing the importance of local ecological conditions (Dozet, 2004; Bijeljac & Sarić, 2005).

Recent trends in dairy production include the addition of medicinal and aromatic plants to cheese to enhance flavor, aroma, nutritional value, and functional properties (Foda et al., 2010; Oraon et al., 2017; Alexa et al., 2018; Sukhikh et al., 2019; El-Sayed & Youssef, 2019; Arkan et al., 2024). Plants can be incorporated into the curd, mixed before molding, or applied to the cheese surface, creating distinctive sensory profiles while preserving traditional characteristics (Josipović et al., 2016; Kalit, 2015). These plants are rich in bioactive compounds such as phenolics, flavonoids, terpenoids, alkaloids, and essential oils, which exhibit antioxidant and antimicrobial activities, acting as natural preservatives and functional ingredients (Maćej et al., 2009; Josipović et al., 2016). Compounds such as carvacrol, thymol, and allicin disrupt microbial membranes, inhibit growth, and prevent oxidative damage, extending shelf life and enhancing health-promoting properties (Foda et al., 2010; Josipović et al., 2016; El-Sayed & Youssef, 2019).

The combination of traditional cheese-making techniques with the addition of selected plants offers an opportunity to produce functional foods while maintaining the authenticity of indigenous products. Understanding the physicochemical effects of such additions is essential to ensure product quality, safety, and sensory appeal. This study aimed to evaluate the influence of selected plant additives on the physicochemical properties of traditional Janj cheese.

MATERIALS AND METHODS

The Janj cheese samples analyzed in this study were prepared in a rural household in the village of Lipovača, Šipovo municipality, an area with a long-standing tradition of producing milk, Janj cream (kajmak), and braided Janj cheese. All analyses were performed at the Faculty of Technology, University of Banja Luka.

A total of twelve cheese samples were produced, divided into two batches of six. Each batch included one control sample without any plant addition. The remaining five samples were enriched with different plants: oregano (*Origanum vulgare*), basil (*Ocimum basilicum*), parsley (*Petroselinum crispum*), rosemary (*Salvia rosmarinus*), and chives (*Allium schoenoprasum*). In the first batch, plants were added at 0.50% of the cheese mass, while in the second batch, the concentration was 1.00% (Table 1). To facilitate uniform incorporation of the plant additives, the traditional braided form was replaced by a rolled shape.

For each batch, 30 L of fresh cow's milk collected during the evening milking was used. The milk was filtered and left at room temperature for approximately 14 hours to achieve optimal conditions for coagulation. Milk readiness was confirmed by heating a 100 mL sample and observing curd formation and whey separation. The milk was then heated to 38 °C, and 60 mL of rennet (0.2% of the total milk volume) was added. The mixture was allowed to rest at this temperature for 20 minutes before raising the temperature to 50 °C. The curd was gently stirred for 10 minutes to facilitate cutting and subsequently

pressed by hand against the container walls for an additional 10 minutes to remove whey. At this stage, the whey temperature reached 48 °C.

After separation, the curd was divided into 500 g portions and flattened to approximately 1 cm thickness. Salt was evenly applied at 1.5%, and plant additives were incorporated according to batch concentration (0.50% or 1.00%). Commercially available dried and crushed plants (KOTANYI GmbH, Wolkersdorf, Austria) were used. The cheese rolls were left on an inclined surface for 30 minutes to allow excess whey to drain, then wrapped in plastic film and stored at +4 °C until further analysis. The production process of Janj cheese is shown in Figure 1.

Table 1. Type and concentration of plants in the tested samples

Plants	Batch 1 (B1)		Batch 2 (B2)	
	Sample	Concentration (%)	Sample	Concentration (%)
-	1.1	-	2.1	-
Oregano (<i>Origanum vulgare</i>)	1.2	0.50	2.2	1.00
Basil (<i>Ocimum basilicum</i>)	1.3	0.50	2.3	1.00
Parsley (<i>Petroselinum crispum</i>)	1.4	0.50	2.4	1.00
Rosemary (<i>Salvia rosmarinus</i>)	1.5	0.50	2.5	1.00
Chives (<i>Allium schoenoprasum</i>)	1.6	0.50	2.6	1.00

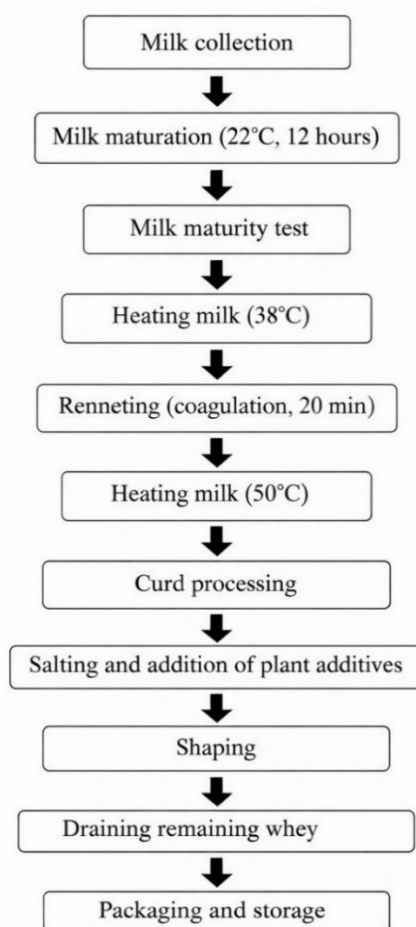


Figure 1. Production flow diagram of Janj cheese

The chemical composition and physicochemical properties of the cheese samples were analyzed, including moisture, ash, protein, fat, chloride content, acidity, pH, and water activity.

Moisture and total solids content were determined by drying the samples at 103 ± 2 °C until a constant weight was achieved, following BAS EN ISO 5534:2006. Ash content was evaluated gravimetrically after incineration in a muffle furnace (Micronal) at 550 °C (Carić et al., 2000). The protein content was calculated from the total nitrogen determined by the Kjeldahl method and converted to crude protein using a conversion factor of 6.38 for milk proteins (BAS EN ISO 8968-1:2015). Fat content was measured using the acid-butyrometric (Gerber) method in accordance with BAS ISO 11870:2012 and BAS ISO 19662:2019.

Chloride levels were determined through potentiometric titration (BAS EN ISO 5943:2015). Water activity (a_w) was measured directly using an AW Meter (Novasina LabMaster-AW 1119971). Cheese acidity was assessed using the Soxhlet–Henkel titration method (Carić et al., 2000). For pH measurement, a cheese solution was prepared by mixing equal parts of cheese and distilled water, and the pH was determined with a digital pH meter (Hanna Instruments, HI 2211) (Carić et al., 2000).

Instrumental color of the cheese was measured in the CIE $L^*a^*b^*$ system using a spectrophotometer CM-2600d (Konica Minolta, Osaka, Japan) on both the outer surface and the cross-sectional area.

Cheese hardness was determined using a Texture Analyser TA.XT plus (Stable Micro Systems, Godalming, UK) by measuring shear force.

Colour and texture measurements were performed ten times per sample, while all other analyses were conducted in triplicate. All obtained data were processed using Microsoft Excel 2013 and IBM SPSS Statistics 22.0 for Windows (Armonk, NY, United States). The results of the analyses are presented as the mean values $\pm$ standard deviation (SD). Differences between arithmetic means were evaluated using one-way analysis of variance (One-way ANOVA) followed by Tukey's HSD post hoc test. Statistical significance was considered at a confidence level of 95% ($p < 0.05$).

RESULTS AND DISCUSSION

The results presented in Table 2 indicate that the plant additives and their concentrations significantly affected ($p < 0.05$) the basic chemical composition of Janj cheese, including water, fat, protein, ash, and chloride content. Such variations are typical for traditional cheeses, where production technology, milk composition, and the addition of plant materials play a key role in shaping the final product characteristics (Fox et al., 2017; Sulejmani et al., 2020; Yerlikaya et al., 2021).

In the first production batch, water content ranged from 53.39% in the sample with 0.5% rosemary addition to 57.72% in the control sample, while the moisture in fat-free matter ranged from 65.11% to 70.38%. The lower water content and moisture in fat-free matter observed in samples with plant additives may be associated with higher dry matter content and potential interactions of plant-derived phenolic compounds with the cheese matrix. Previous studies have shown that the addition of plants such as oregano and rosemary can increase the antioxidant capacity and phenolic content in cheeses, which may indirectly contribute to the stabilization of the protein network (Yerlikaya et al., 2021). In the second batch, water content was lower, ranging from 48.96% (rosemary, 1.0%) to 51.31% (basil, 1.0%), which corresponded with higher dry matter and a more compact cheese texture. These results confirm that plant additives modify the physicochemical properties of cheeses, including moisture and structure, in line with the findings of Sulejmani et al. (2020).

Fat content in the first batch was uniform (18.0%), while in the second batch it was higher, ranging from 22.0% to 22.5%, which directly affected the values of fat in dry matter (FDM). In the first batch, FDM ranged from 38.62% in the sample with 0.5% rosemary to 42.57% in the control, while higher values were recorded in the second batch, with a maximum of 45.18% in the sample with 1.0% basil. These differences confirm that technological and production batch factors have a dominant effect on the lipid composition of cheese, greater than the effect of the plant additive itself.

Protein content showed marked differences between samples. In the first batch, the control sample had the lowest protein content (18.12%), while the sample with 0.5% rosemary had the highest value (25.05%). A

similar trend was observed in the second batch, where samples with plant additives had protein contents ranging from 21.04% to 23.95%, compared to 23.37% in the control. The higher protein content in samples with lower moisture is primarily due to dry matter concentration. Additionally, phenolic compounds from the added plants may potentially support protein stability, as suggested by their effect on antioxidant capacity reported in previous studies (Yerlikaya et al., 2021; Sulejmani et al., 2020).

Ash content was significantly higher in samples with plant additives compared to the controls. In the first batch, ash content ranged from 1.85% in the control sample to 2.89% in the sample with oregano, while in the second batch, values ranged from 2.30% to 2.74%. This increase in total ash indicates that aromatic plants contribute to enhancing the mineral composition of cheeses, including macro- and microelements, as supported by previous studies on plant-enriched cheeses (Savanović et al., 2022; Savanović et al., 2023a).

Chloride content showed significant differences between samples. In the first batch, values ranged from 0.60% in the sample with rosemary to 1.62% in the sample with oregano, while in the second batch, values ranged from 0.75% to 1.44%. These differences suggest that plant additives may influence salt diffusion and binding within the cheese matrix, which may be associated with changes in the protein matrix influenced by the presence of aromatic plants (Yerlikaya et al., 2021; Sulejmani et al., 2020).

Comparative analysis of production batches revealed that the second batch had lower water content and higher fat and protein content, indicating a more compact structure and higher nutritional value. Although plant additives modify the chemical composition of Janj cheese, technological and temporal production factors play a determining role in the final product characteristics (Walstra et al., 2006; Fox et al., 2017).

Table 2. Mean values of the basic chemical composition of the Janj cheese samples

Batch	Sample	Moisture content (%)	Moisture in fat-free matter (%)	Fat (%)	Fat in dry matter (%)	Protein (%)	Ash (%)	Chloride (%)
B1	1.1	57.72 ^f ± 0.10	70.38 ^f ± 0.12	18.00 ± 0.00	42.57 ^f ± 0.10	18.12 ^a ± 0.20	1.85 ^a ± 0.05	0.78 ^b ± 0.04
	1.2	54.89 ^c ± 0.10	66.94 ^c ± 0.11	18.00 ± 0.00	39.90 ^c ± 0.08	21.59 ^c ± 0.06	2.89 ^d ± 0.26	1.62 ^c ± 0.00
	1.3	54.35 ^b ± 0.27	66.28 ^b ± 0.32	18.00 ± 0.00	39.43 ^b ± 0.23	23.05 ^c ± 0.22	2.38 ^c ± 0.01	1.24 ^c ± 0.03
	1.4	56.92 ^c ± 0.33	69.42 ^c ± 0.41	18.00 ± 0.00	41.79 ^c ± 0.32	20.81 ^b ± 0.15	2.09 ^b ± 0.04	0.80 ^b ± 0.01
	1.5	53.39 ^a ± 0.28	65.11 ^a ± 0.23	18.00 ± 0.00	38.62 ^a ± 0.16	25.05 ^f ± 0.16	2.17 ^{bc} ± 0.06	0.60 ^a ± 0.05
	1.6	55.93 ^d ± 0.23	68.21 ^d ± 0.28	18.00 ± 0.00	40.85 ^d ± 0.21	22.15 ^d ± 0.20	2.34 ^c ± 0.03	1.50 ^d ± 0.01
B2	2.1	49.27 ^{ab} ± 0.06	63.17 ^a ± 0.08	22.00 ± 0.00	43.37 ^a ± 0.06	23.37 ^c ± 0.11	2.30 ^a ± 0.04	0.78 ^a ± 0.01
	2.2	49.29 ^{ab} ± 0.38	63.19 ^a ± 0.49	22.00 ± 0.00	43.38 ^a ± 0.33	23.95 ^d ± 0.03	2.45 ^b ± 0.00	0.86 ^{ab} ± 0.01
	2.3	51.31 ^d ± 0.22	65.79 ^d ± 0.28	22.00 ± 0.00	45.18 ^d ± 0.20	21.04 ^a ± 0.05	2.34 ^a ± 0.01	0.97 ^b ± 0.10
	2.4	49.49 ^b ± 0.14	63.86 ^b ± 0.18	22.50 ± 0.00	44.54 ^c ± 0.12	22.06 ^b ± 0.23	2.65 ^c ± 0.02	1.26 ^c ± 0.09
	2.5	48.96 ^a ± 0.20	63.35 ^{ab} ± 0.00	22.50 ± 0.00	44.08 ^b ± 0.17	23.20 ^c ± 0.48	2.40 ^{ab} ± 0.01	0.75 ^a ± 0.03
	2.6	50.41 ^c ± 0.33	64.63 ^c ± 0.42	22.00 ± 0.00	44.37 ^{bc} ± 0.30	22.42 ^b ± 0.25	2.74 ^d ± 0.06	1.44 ^d ± 0.08

(a–f) Different letters within the same column and batch indicate statistically significant differences among samples at $p < 0.05$.

The addition of selected plant additives to Janj cheese significantly influenced the physicochemical properties, including pH, titratable acidity, and water activity. Similar trends have been reported in studies on fresh cream cheese, where the incorporation of spices such as oregano, basil, parsley, rosemary, and chives resulted in statistically significant changes ($p < 0.05$) in these parameters (Savanović et al., 2023b). These findings suggest that plant additives can modulate cheese characteristics while maintaining the overall quality and safety of the product, supporting their potential use in traditional cheese production. As shown in Table 3, the pH, titratable acidity, and water activity (a_w) of the Janj cheese samples varied among samples within each batch depending on the type of plant additive used. The pH values of the tested Janj cheese samples ranged from 5.00 to 5.26 in the first batch and from 5.06 to 5.51 in the second batch. These values are consistent with the typical pH ranges of semi-hard and traditional cheeses, where the final pH reflects the balance between lactic acid production by lactobacilli and its consumption during ripening (Trmčić et al., 2017). Lower pH (higher acidity) generally indicates more intensive lactose fermentation and lactic acid accumulation, while higher pH reflects less pronounced acidification.

Titratable acidity ($^{\circ}\text{SH}$) in the first batch varied between 47.20 and 53.60 $^{\circ}\text{SH}$, whereas in the second batch it was significantly lower (28.00–34.20 $^{\circ}\text{SH}$), inversely related to pH values (lower pH = higher acidity). This phenomenon is typical, as pH and titratable acidity represent two sides of the same chemical and microbiological dynamics during cheese ripening, with pH decreasing while titratable acidity increases due to lactobacilli activity and lactic acid production (Makarijowski and Najdovski, 2025).

Water activity (a_w) is a physicochemical parameter indicating the availability of free water for microbiological and biochemical processes (CDR, 2024). In the first batch, a_w values were slightly higher (0.9200–0.9320), while in the second batch they were somewhat lower (0.9010–0.9155). Water activity depends not only on the total water content but also on the concentration of dissolved substances such as salts and proteins, as well as the structure of the cheese matrix (Hickey et al., 2013). Lower a_w indicates stronger water binding in the cheese, typically achieved with higher dry matter and salt content, corresponding to a firmer structure and less free water in samples with higher protein and lower water content.

Variations in a_w values between samples may be associated with differences in chemical composition (salts, proteins, ash), microstructure, and ripening stage, as changes in the protein matrix and salt content can influence the distribution of free water in the cheese matrix (Hickey et al., 2013). Both pH and water activity (a_w) are key factors affecting the microbial safety of cheese, as lower pH and a_w values restrict the growth of pathogenic and spoilage microorganisms, thereby enhancing product safety (CDR, 2024).

Table 3. Mean values of pH, titratable acidity, and water activity (a_w) of the Janj cheese samples

Batch	Sample	pH	Titratable acidity ($^{\circ}\text{SH}$)	a_w
B1	1.1	5.18 ^b ± 0.06	53.60 ^c ± 1.13	0.9320 ^c ± 0.001
	1.2	5.26 ^c ± 0.02	50.80 ^{bc} ± 0.56	0.9175 ^a ± 0.001
	1.3	5.03 ^a ± 0.03	52.40 ^{dc} ± 0.56	0.9255 ^b ± 0.002
	1.4	5.24 ^{bc} ± 0.05	51.60 ^{cd} ± 0.56	0.9250 ^b ± 0.001
	1.5	5.18 ^b ± 0.03	50.00 ^b ± 0.56	0.9240 ^b ± 0.000
	1.6	5.00 ^a ± 0.06	47.20 ^a ± 1.13	0.9200 ^a ± 0.001
B2	2.1	5.51 ^c ± 0.05	30.00 ^b ± 0.56	0.9075 ^b ± 0.001
	2.2	5.40 ^d ± 0.07	28.00 ^a ± 0.00	0.9155 ^d ± 0.001
	2.3	5.23 ^b ± 0.03	33.80 ^{cd} ± 0.28	0.9145 ^d ± 0.001
	2.4	5.26 ^{bc} ± 0.03	33.40 ^c ± 0.28	0.9100 ^c ± 0.000
	2.5	5.06 ^a ± 0.09	34.20 ^d ± 0.28	0.9105 ^c ± 0.001
	2.6	5.35 ^{cd} ± 0.05	30.00 ^b ± 0.56	0.9010 ^a ± 0.000

(a–e) Different letters within the same column and batch indicate statistically significant differences among samples at $p < 0.05$.

Instrumental color analysis of the cheeses was performed using the CIELAB system, where L* indicates lightness (0 = black, 100 = white), a* represents the green–red component, and b* describes the blue–yellow component (Table 4). This system allows for objective measurement of visual properties and differentiation between samples, which is crucial for evaluating product quality and uniformity (Póltorak et al., 2015; Minz & Saini, 2021). Instrumental color measurements showed significant differences between the samples within each batch ($p < 0.05$). L* values in the first batch ranged from 88.88 to 92.30 (on the external surface), while in the second batch they ranged from 85.75 to 88.93. Differences in surface and cross-sectional lightness can be attributed to the presence of plant additives, variations between production batches, and differences in dry matter content. The a* and b* components exhibited lower variability but indicated a predominance of green hues (negative a* values) and yellow tones (positive b* values), consistent with the characteristics of dairy pigments and the influence of additives such as aromatic plants (Póltorak et al., 2015; Minz & Saini, 2021). Elevated b* values in samples containing plant additives may reflect interactions between plant pigments and the protein–fat matrix of the cheese, contributing to a richer yellow hue.

Table 4. Mean values of color parameters (L, a, b*) and hardness of the Janj cheese samples

Batch	Sample	Surface color			Cross-section color			Hardness (kg)
		L*	a*	b*	L*	a*	b*	
B1	1.1	92.30 ^d ± 0.44	−0.84 ^c ± 0.08	13.68 ^a ± 0.40	90.91 ^b ± 0.41	−0.53 ^{cd} ± 0.11	13.46 ^a ± 0.44	0.1794 ^a ± 0.04
	1.2	88.88 ^a ± 1.28	−1.03 ^{abc} ± 0.15	17.01 ^d ± 1.58	80.76 ^a ± 6.97	−0.12 ^d ± 0.71	16.26 ^b ± 1.92	0.3634 ^c ± 0.04
	1.3	89.58 ^{ab} ± 0.38	−1.08 ^{ab} ± 0.09	16.30 ^{cd} ± 0.50	84.59 ^{ab} ± 4.06	−0.71 ^{bcd} ± 0.26	15.26 ^{ab} ± 1.02	0.3422 ^c ± 0.07
	1.4	91.22 ^c ± 0.34	−0.92 ^{bc} ± 0.16	14.31 ^{ab} ± 0.61	82.69 ^{ab} ± 5.12	−1.16 ^a ± 0.61	15.28 ^{ab} ± 1.89	0.2213 ^{ab} ± 0.07
	1.5	89.10 ^a ± 0.41	−1.12 ^a ± 0.14	16.86 ^d ± 0.73	87.09 ^{ab} ± 1.08	−0.90 ^{abc} ± 0.24	16.01 ^b ± 1.26	0.3938 ^c ± 0.04
	1.6	90.51 ^{bc} ± 0.85	−1.08 ^{ab} ± 0.15	15.35 ^{bc} ± 0.66	81.90 ^a ± 9.46	−1.27 ^{ab} ± 0.55	15.24 ^{ab} ± 2.01	0.2576 ^b ± 0.07
B2	2.1	87.93 ^a ± 1.09	−0.54 ^a ± 0.26	17.98 ^a ± 1.12	87.98 ^b ± 1.01	−0.002 ^{ab} ± 0.17	19.09 ^b ± 0.89	0.4676 ^{ab} ± 0.08
	2.2	88.93 ^a ± 0.72	−0.12 ^c ± 0.16	18.71 ^a ± 1.12	75.02 ^a ± 12.28	−0.44 ^b ± 1.71	17.07 ^{ab} ± 2.56	0.5264 ^b ± 0.09
	2.3	88.65 ^a ± 1.81	−0.14 ^{bc} ± 0.16	17.83 ^a ± 1.76	84.38 ^{ab} ± 6.57	−0.10 ^{ab} ± 0.51	17.19 ^{ab} ± 1.35	0.3733 ^a ± 0.10
	2.4	88.72 ^a ± 1.79	−0.12 ^c ± 0.12	18.46 ^a ± 2.53	73.71 ^a ± 13.23	−1.15 ^a ± 0.78	15.62 ^a ± 2.61	0.5686 ^b ± 0.10
	2.5	85.75 ^a ± 5.15	−0.41 ^{ab} ± 0.20	18.51 ^a ± 0.83	83.81 ^{ab} ± 5.56	−0.11 ^{ab} ± 0.59	18.27 ^{ab} ± 2.09	0.5064 ^b ± 0.11
	2.6	88.80 ^a ± 1.34	−0.18 ^{bc} ± 0.19	18.40 ^a ± 1.09	82.98 ^{ab} ± 5.45	−0.67 ^{ab} ± 0.35	18.73 ^b ± 1.60	0.5312 ^b ± 0.09

(a–d) Different letters within the same column and batch indicate statistically significant differences among samples at $p < 0.05$.

Instrumental hardness showed significant differences ($p < 0.05$) among samples and batches (Table 4). In Batch 1, the sample with oregano exhibited the highest hardness (0.3634 kg), whereas the control sample had the lowest value (0.1794 kg). In Batch 2, hardness was generally higher (0.4676–0.5686 kg), likely reflecting reduced moisture content, increased dry matter, and potential differences in protein and fat structure caused by the production batch and the presence of plant additives. Cheese hardness is directly related to the microstructure of the protein matrix, textural cohesiveness, and sensory perception of firmness (Blázquez et al., 2006).

Differences in color and hardness between batches and samples confirm that technological factors and additives can significantly modulate the visual and textural properties of Janj cheese, and instrumental measurements provide a precise, objective, and reproducible method for quantifying these attributes. Instrumental color and hardness measurements correlate with sensory descriptions and allow for the prediction of qualitative characteristics perceived visually and tactually by consumers (Póltorak et al., 2015; Minz & Saini, 2021; Blázquez et al., 2006).

The present study demonstrated that the addition of selected plant additives significantly influenced the physicochemical properties of Janj cheese. These changes are consistent with previous reports indicating that the incorporation of natural plant additives can modify the compositional and functional attributes of dairy products. Moreover, previous studies have demonstrated that such plant additives enhance the biological properties of Janj cheese, including its antioxidant and antimicrobial activities (Savanović et al., 2025a). In addition, the sensory evaluation of Janj cheese revealed that plant supplementation positively affected taste, aroma, color, and texture, with the highest acceptability observed for cheeses enriched with oregano and chives (Savanović et al., 2025b). Taken together, these findings suggest that the strategic use of natural plant additives not only improves the functional and bioactive qualities of Janj cheese but also increases its overall consumer appeal, supporting the integration of traditional dairy products with functional ingredients for enhanced nutritional and sensory value.

CONCLUSIONS

The addition of selected plant additives significantly affected the physicochemical characteristics of traditional Janj cheese, influencing moisture, dry matter, fat, protein, ash, sodium chloride content, pH, titratable acidity, water activity, color, and texture. These findings demonstrate that plant additives can effectively modulate the composition and structural properties of cheese while preserving its authentic traditional qualities. The study underscores the importance of careful formulation and precise technological control in artisanal cheese production, highlighting how such interventions can support product consistency and quality. This knowledge contributes to maintaining the cultural and economic value of traditional cheeses and provides a scientific basis for potential optimization of their production.

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REFERENCES

- Alexa, E., Danciu, C., Cocan, I., Negrea, M., Morar, A., Obistioiu, D., Dogaru, D., Berbecea, A., & Radulov, I. (2018). Chemical composition and antimicrobial potential of *Satureja hortensis* L. in fresh cow cheese. *Journal of Food Quality*, 8424035. <https://doi.org/10.1155/2018/8424035>
- Arkan, N. D., Setyawardani, T., Sumarmono, J., Naufalin, R., Santosa, S. S., & Rahardjo, A. H. D. (2024). Effect of spice powder on physicochemical characteristics, functional properties and microbiological quality in soft cheese. *Asian Journal of Dairy and Food Research*, 43(3), 396–403. <https://doi.org/10.18805/ajdfr.DRF-374>
- BAS EN ISO 5534:2006. Cheese and processed cheese - Determination of total solids content (Reference method). Institute for Standardization of Bosnia and Herzegovina.
- BAS EN ISO 5943:2015. Cheese and processed cheese products - Determination of chloride content - Potentiometric titration method. Institute for Standardization of Bosnia and Herzegovina.
- BAS EN ISO 8968-1:2015. Milk and milk products - Determination of nitrogen content - Part 1: Kjeldahl principle and crude protein calculation. Institute for Standardization of Bosnia and Herzegovina.
- BAS ISO 11870:2012. Milk and milk products - Determination of fat content - General guidance on the use of butyrometric methods. Institute for Standardization of Bosnia and Herzegovina.

- BAS ISO 19662:2019. Milk - Determination of fat content - Acido-butyrometric (Gerber method). Institute for Standardization of Bosnia and Herzegovina.
- Bijeljic, S., Sarić, Z. (2005). *Autohtoni mliječni proizvodi sa osnovama sirarstva [Autochthonous Dairy Products with Fundamentals of Cheesemaking]*. Sarajevo: Poljoprivredni fakultet.
- Blázquez, C., Downey, G., O’Callaghan, D., Howard, V., Delahunty, C., Sheehan, E., Everard, C., O’Donnell, C. P. (2006). Modelling of sensory and instrumental texture parameters in processed cheese by near infrared reflectance spectroscopy. *Journal of Dairy Research*, 73(1), 58–69. <https://doi.org/10.1017/S0022029905001536>
- Cardin, M., Cardazzo, B., Mounier, J., Novelli, E., Coton, M., & Coton, E. (2022). Authenticity and typicity of traditional cheeses: A review on geographical origin authentication methods. *Foods*, 11(21), 3379. <https://doi.org/10.3390/foods11213379>
- Carić, M., Milanović, S., & Vucelja, D. (2000). *Standardne metode analize mleka i mlečnih proizvoda [Standard Methods for the Analysis of Milk and Dairy Products]*. Novi Sad: Tehnološki fakultet.
- Center for Dairy Research (CDR). (2024). Understanding water activity and cheese safety. Center for Dairy Research.
- Dozet, N. (2004). *Travničko-Vlašićki sir, sirevi u salamuri [Travnik–Vlašić Cheese, Brined Cheeses]*. Srpsko Sarajevo: Poljoprivredni fakultet Univerziteta u Srpskom Sarajevu.
- Dozet, N., & Maćej, O. (2006). *Autohtoni beli sirevi u salamuri [Native white cheeses in brine]*. Beograd: Univerzitet u Beogradu, Poljoprivredni fakultet.
- El-Sayed, S. M., & Youssef, A. M. (2019). Potential application of herbs and spices and their effects in functional dairy products. *Heliyon*, 5(6), e01989. <https://doi.org/10.1016/j.heliyon.2019.e01989>
- Foda, M. I., El-Sayed, M. A., Hassan, A. A., Rasmy, N. M., & El-Moghazy, M. M. (2010). Effect of spearmint essential oil on chemical composition and sensory properties of white cheese. *Journal of American Science*, 6(5), 272–279.
- Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2017). *Fundamentals of cheese science* (2nd ed.). Springer. <https://doi.org/10.1007/978-1-4899-7681-9>
- Hickey, D.K., Guinee, T.P., Hou, J., & Wilkinson, M.G. (2013). Effects of variation in cheese composition and maturation on water activity in Cheddar cheese during ripening. *International Dairy Journal*, 30(1), 53-58. <https://doi.org/10.1016/j.idairyj.2012.11.006>
- Josipović, R., Markov, K., Frece, J., Stanzer, D., Cvitković, A., & Mrvčić, J. (2016). The use of spices in the production of traditional cheeses. *Mljekarstvo*, 66(1), 12–25. <https://doi.org/10.15567/mljekarstvo.2016.0102>
- Kalit, S. (2015). Opće sirarstvo [General cheesemaking]. In B. Matijević (Ed.), *Sirarstvo u teoriji i praksi [Cheesemaking in theory and practice]* (pp. 29–45). Karlovac, Hrvatska: Veleučilište u Karlovcu.
- Keskin, E., & Dağ, T. (2020). Identity of cheese: A research on the cheeses of the Aegean region in Turkey. *Journal of Ethnic Foods*, 7, 25. <https://doi.org/10.1186/s42779-020-00062-4>
- Maćej, O., Seratlić, S., Jovanović, S., Radin, D., Vučić, T., & Miloradović, Z. (2009). Karakteristike sireva sa dodatkom lekovitog i aromatičnog bilja. *Food Industry*, 1-2, 123-129.
- Makarijoski, B., & Najdovski, Z. (2025). Monitoring of active and titratable acidity in white-brined cheese during ripening period. *Journal of Agriculture and Plant Sciences*, 22(2), 39-43. <https://doi.org/10.46763/JAPS2422239m>
- Minz, P. S., & Saini, C. S. (2021). Comparison of computer vision system and colour spectrophotometer for colour measurement of mozzarella cheese. *Applied Food Research*, 1(2), 100020. <https://doi.org/10.1016/j.afres.2021.100020>
- Montel, M. C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D. A., Desmasures, N., & Berthier, F. (2014). Traditional cheeses: Rich and diverse microbiota with associated benefits. *International Journal of Food Microbiology*, 177, 136–154. <https://doi.org/10.1016/j.ijfoodmicro.2014.02.019>

- Oraon, L., Atanu, J., Prajapati, P. S., & Suvera, P. (2017). Application of herbs in functional dairy products – a review. *Journal of Dairy, Veterinary and Animal Research*, 5(3), 109–115. <https://doi.org/10.15406/jdvar.2017.05.00145>
- Póltorak, A., Wyrwisz, J., Moczowska, M., Marcinkowska-Lesiak, M., Stelmasiak, A., Ulanicka, U., Zalewska, M., & Wierzbicka, A. (2015). Correlation between instrumental texture and colour quality attributes with sensory analysis of selected cheeses as affected by fat contents. *International Journal of Food Science & Technology*, 50(4), 999–1008. <https://doi.org/10.1111/ijfs.12707>
- Popović-Vranješ, A. (2015). *Specijalno sirarstvo [Specialized Cheesemaking]*. Novi Sad: Poljoprivredni fakultet Univerziteta u Novom Sadu.
- Savanović, D., Velemir, A., Savić, A., Topalić Trivunović Lj., Savanović, J., & Muždeka, V. (2025a). Effect of selected plant species on the antioxidant and antimicrobial properties of Janj cheese. *STED Journal*, 7(2), 8-19. <https://doi.org/10.63395/STEDJournal0702081S8>
- Savanović, D., Velemir, A., Savanović, J., & Sekulić, J. (2023a). Content of macro- and microelements in cheeses produced with the addition of different spices. *Journal of Chemists, Technologists and Environmentalists*, 4(1), 7–13. <https://doi.org/10.59919/JCTE04202301E235>
- Savanović, D., Velemir, A., Savanović, J., Savić, A., & Babić, D. (2023b). Physicochemical properties of cream cheese with the addition of selected spices. *XII International Conference on Social and Technological Development, STED 2023, Proceedings*, 396–402. University PIM, Banja Luka.
- Savanović, D., Velemir, A., Savić, A., Savanović, J., & Sekulić, J. (2022). Influence of addition of selected spices on mineral composition of fresh cream cheese. XI International conference of social and technological development, *STED 2022, Proceedings*, 547-554. University PIM, Banja Luka.
- Savanović, D., Velemir, A., Savić, A., Savanović, J., Šojić, B., & Muždeka, V. (2025b). Sensory properties of janj cheese with the addition of selected plant species. *XIV International Conference on Social and Technological Development, STED 2025, Proceedings*, 231-238. University PIM, Banja Luka.
- Sukhikh, S. A., Astakhova, L. A., Golubcova, Y. V., Lukin, A. A., Proseкова, E. A., Milent'eva, I. S., et al. (2019). Functional dairy products enriched with plant ingredients. *Foods and Raw Materials*, 7(2), 428–438. <https://doi.org/10.21603/2308-4057-2019-2-428-438>
- Sulejmani, E., Sahingil, D., & Hayaloglu, A. A. (2020). A comparative study of compositional, antioxidant capacity, ACE-inhibition activity, RP-HPLC peptide profile and volatile compounds of herbal artisanal cheeses. *International Dairy Journal*, 111, 104837. <https://doi.org/10.1016/j.idairyj.2020.104837>
- Trmčić, A., Ralyea, R., Meunier-Goddik, L., Donnelly, C., Glass, K., D'Amico, D., Meredith, E., Kehler, M., Tranchina, N., McCue, C., & Wiedmann, M. (2017). Consensus categorization of cheese based on water activity and pH — A rational approach to systemizing cheese diversity. *Journal of Dairy Science*, 100(1), 841-847. <https://doi.org/10.3168/jds.2016-11621>
- Vujko, A., Arsić, M., & Bojović, R. (2025). From Local Product to Destination Identity: Leveraging Cave-Aged Cheese for Sustainable Rural Tourism Development. *Agriculture*, 15(11), 1137. <https://doi.org/10.3390/agriculture15111137>
- Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2006). *Dairy science and technology* (2nd ed.). CRC Press.
- Yerlikaya, O., Akan, E., Bayram, O. Y., Karaman, A. D., & Kinik, O. (2021). The impact of medicinal and aromatic plant addition on antioxidant, total phenolic, antimicrobial activities, and microbiological quality of Mozzarella cheese. *International Food Research Journal*, 28(3), 508–516. <https://doi.org/10.47836/ifrj.28.3.10>

Thermal processing of finely comminuted cooked sausages: Influence of diameter and casing type

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Abstract

The objective of this study was to investigate the influence of sausage diameter and the casing type on the thermal processing parameters of finely comminuted cooked sausages. Thermal treatment plays a vital role in extending the shelf life and ensuring the safety of cooked sausages. During thermal processing, the product must reach a core temperature of at least 72°C to achieve a safe, high-quality, and stable product, maintaining desirable sensory properties such as color, aroma, and texture. The experimental part of this research involved producing three batches of chicken-based hot dogs with identical formulations but varying diameters and different casing types (22 mm with polyamide casings - PAC22, and 10 mm and 22 mm cellulose casings - CC10 and CC22). The process began with drying and smoking (both CC samples), followed by thermal processing at 75-80°C until the core temperature reached 72°C (all samples), and finished with a cooling phase. The cooking phase, as a part of the thermal process, was continuously monitored using probe thermometers. Results showed that the product's diameter significantly influenced the time required to reach the target internal temperature. Total process durations were 115.7 min (CC10), 141.3 min (CC22), and 129.3 min (PAC22), with corresponding cooking phase durations of 100, 111.7, and 101 minutes, respectively. Sausages with larger diameters needed longer heating periods due to slower heat penetration. In addition, the type of casing influenced heat transfer efficiency and moisture retention. The findings emphasize the importance of continuous temperature monitoring and precise control during processing to ensure both product safety and technological quality.

Keywords: sausage diameter, casing type, thermal processing

INTRODUCTION

The quality of meat products is primarily determined by the quality of raw materials, the type and concentration of additives and spices used to improve organoleptic and technological properties, the selected processing method, preservation techniques, and packaging materials and methods (Lee et al., 2023). Cooked sausages belong to one of the widest categories of processed meat products, and their manufacture involves a complex series of biochemical, chemical, and physical transformations (Zhang et al., 2024).

Due to its high susceptibility to spoilage, meat is classified as a microbiologically sensitive food product. During handling and processing, it is particularly susceptible to microbial contamination, which requires the use of appropriate preservation methods to extend its shelf life (Vuković, 2012). In meat technology, preservation approaches are generally categorized as physical (chilling, freezing, heat treatment, drying, and lyophilization), chemical (salting, curing, smoking, modified atmosphere packaging, and the use of permitted preservatives), and biological (the use of selected microorganisms whose metabolic activity inhibits the growth of undesirable microflora) (Watson, 2025).

Finely comminuted cooked sausages, including frankfurters, wieners, bologna-type sausages, and similar products, are produced from various types of meat, fatty tissue, connective tissue, water, salt, additives, spices, carbohydrates, and protein preparations. Their distinguishing characteristic is a homogeneous meat emulsion with no visible pieces of muscle, fat, or connective tissue. This emulsion, comprising 65–80% of the total batter, undergoes profound structural transformation during heat treatment, as protein denaturation converts the soft, fluid emulsion into a firm, sliceable product (Zhu et al., 2020; Cheng & Sun, 2008; Sante-Lhoutellier et al., 2008). At the molecular level, the gelation of myofibrillar proteins, primarily myosin, which begins to denature at 40°C and undergoes complete coagulation above 53°C is central to the development of the characteristic firm, elastic texture of the final product (Strzelczak et al., 2021).

The primary function of casings is to maintain the product in the shape and size most suitable for subsequent processing steps and retail handling. Casings must permit water evaporation from the batter and, in applicable product types, allow smoke penetration, while simultaneously being impermeable to fat migration. These requirements demand that casings be sufficiently elastic, strong, non-hygroscopic, and resistant to microbial degradation. For smoked products, casings must also be permeable to gases and moisture (Savić & Savić, 2016). In today's sausage production, various types of casings are employed - natural, manufactured collagen, cellulose, plastic, and co-extruded casings - with the two most important physical properties being their barrier characteristics and mechanical strength, both of which strongly affect consumer perception of bite, snap, and flavor (Suurs & Barbut, 2020).

Sausage casing selection is of critical importance, as it directly influences the integrity, size, and shape of the final product, while simultaneously facilitating the transformation of the soft, fluid meat batter into the desired semi-firm product (Savić & Savić, 2016). The two most important physical characteristics of casings are their barrier properties and mechanical strength, both of which significantly influence consumer perception of texture and flavor (Suurs & Barbut, 2020). Both natural and artificial casings are used in sausage manufacture, though artificial casings have become predominant due to their uniformity, sterilizability, and consistent dimensions. Cellulose casings, widely used for frankfurter-type sausages, are permeable to water vapor and smoke unless coated with plastic, thereby enabling the characteristic smoke color and flavor of the final product to develop (Barbut, 2024; Suurs & Barbut, 2020).

Thermal processing constitutes a critical control point in the production of cooked sausages and typically consists of sequential phases: surface drying, smoking, and pasteurization by immersion in hot water or steam. The drying phase is conducted at 40–60°C, with open air-exchange dampers to facilitate surface moisture removal and optimize smoke adsorption onto the casing. Hot smoking is subsequently performed at 60–70°C until a characteristic golden-brown color is achieved. Smoking confers desirable sensory properties - color, aroma, and flavor - while also contributing to preservation through the antimicrobial and antioxidant activity of phenolic smoke components such as guaiacol, cresols, and catechol (Nizio et al., 2023). The relative humidity within the smoking chamber should be maintained at approximately 65–75%, as humidity significantly influences the uniformity and rate of smoke deposition (Vuković, 2012).

After smoking, the sausages are pasteurized in a humid environment at 75–80°C, with the precise temperature selected based on product diameter. The critical food safety criterion is that a minimum internal (core) temperature of 72°C must be achieved and maintained throughout the product (Rašeta et al., 2025; Rašeta et al., 2023; Vuković, 2012). This thermal treatment is sufficient to inactivate the vegetative forms of most spoilage and pathogenic microorganisms, achieving a > 6-log reduction in *Listeria monocytogenes*; however, temperatures in the 75–80°C range are insufficient to reduce heat-resistant spore-forming bacteria, including species of *Bacillus* and *Clostridium*. This inherent limitation of pasteurization-based heat treatment, combined with the compositional characteristics of cooked sausages, restricts their thermostability and necessitates cold chain management throughout distribution (Rašeta et al., 2025).

Cooked sausages exhibit a restricted shelf life when stored at temperatures below 4°C. Shelf life can be substantially extended by filling products into casings that are permeable to water vapor and smoke or by vacuum packaging and packaging under a modified atmosphere of carbon dioxide (Vuković, 2012). The combination of heat-smoking treatment provides a synergistic preservation effect, as both processes

act independently yet complementarily to reduce the product microbial load and water activity (Nizio et al., 2023).

This study aims to investigate critical technological parameters during the thermal processing of finely comminuted cooked sausages, with a particular focus on producing a safe, commercially stable product, and highlights the importance of consistent, continuous, and reliable monitoring of thermal processing parameters.

MATERIALS AND METHODS

The complete production process is illustrated in Figure 1. Three samples of chicken-based hot dogs with identical composition but differing in casing type and diameter were produced for this study. The duration of thermal processing needed to reach a safe core temperature was monitored using calibrated needle-probe thermometers inserted into the thermal center of the product. For each product variant, thermal processing parameters were monitored across three independently produced batches.

All samples were manufactured from mechanically deboned chicken meat (MDM) as the principal raw material, supplemented with chicken skin emulsion, water, and the following functional ingredients: soy proteins, potato starch, dietary plant fibre, sodium chloride, spices and spice extracts, stabilisers (E450, E452), thickener (E407), antioxidant (E301), flavor enhancer (E621), and preservative (E250). The chicken meat fraction constituted 82% of the total formulation.

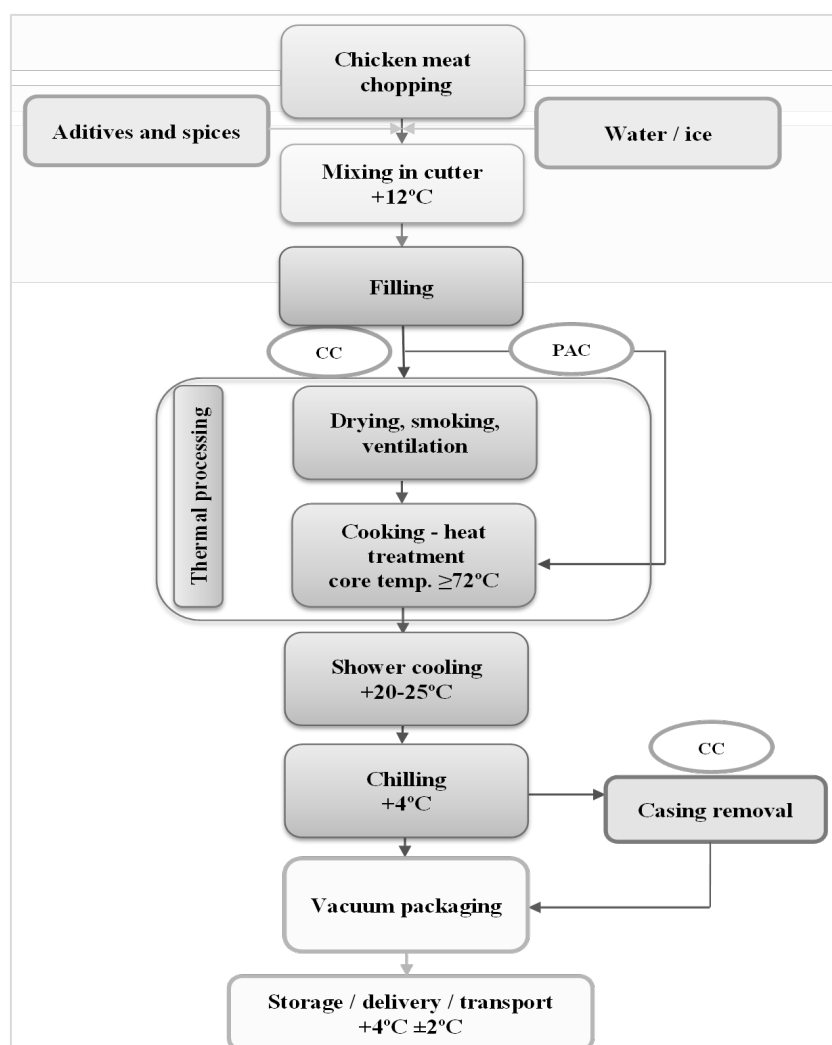


Figure 1. Process flow diagram

Batter preparation was carried out in a cutter. The prescribed quantity of MDM was first comminuted, followed by the sequential addition of polyphosphates, preservatives, and chilled water. After a defined mixing interval, the chicken skin emulsion and the remaining spice-additive blend were incorporated. Chopping was continued until the batter reached an internal temperature of 12°C, at which point further chopping was terminated to prevent fat-phase destabilisation. The prepared batter was transferred to a sausage filler and stuffed into the respective casings (cellulose or polyamide) to a target length. The filled products were loaded onto sticks, suspended on smoking frames, and transported to the processing chamber.

Table 1. Overview of experimental batches

Sample	Casing Type	Diameter (mm)	Thermal Treatment
PAC22	Polyamide casing	Ø 22	Cooking
CC22	Cellulose casing (peelable)	Ø 22	Drying/smoking/cooking
CC10	Cellulose casing (peelable)	Ø 10	Drying/smoking/cooking

Thermal processing was conducted in a climate-controlled processing chamber. Sample PAC22 (polyamide casing) underwent steam cooking until a minimum core temperature of 72°C was reached, without a smoking phase, as polyamide casings are impermeable to smoke. The thermal treatment for samples CC22 and CC10 (cellulose casing) consisted of combined drying, smoking and cooking phases: initial drying at 60-65 °C, then brief smoking at 70 °C, and finally steam cooking until a minimum core temperature of 72 °C was reached. After completion of thermal processing, products were shower-cooled with cold water, then subjected to forced-air cooling until the core temperature fell below 4°C. For both CC samples, the peelable cellulose casings were removed mechanically after cooling. All samples were vacuum-packaged on an inline vacuum packaging line in pre-determined portion sizes.

RESULTS AND DISCUSSION

The graphical representation of mean thermal processing phase durations (Figure 2) provides a clear visual basis for comparing the three frankfurter variants investigated in this study.

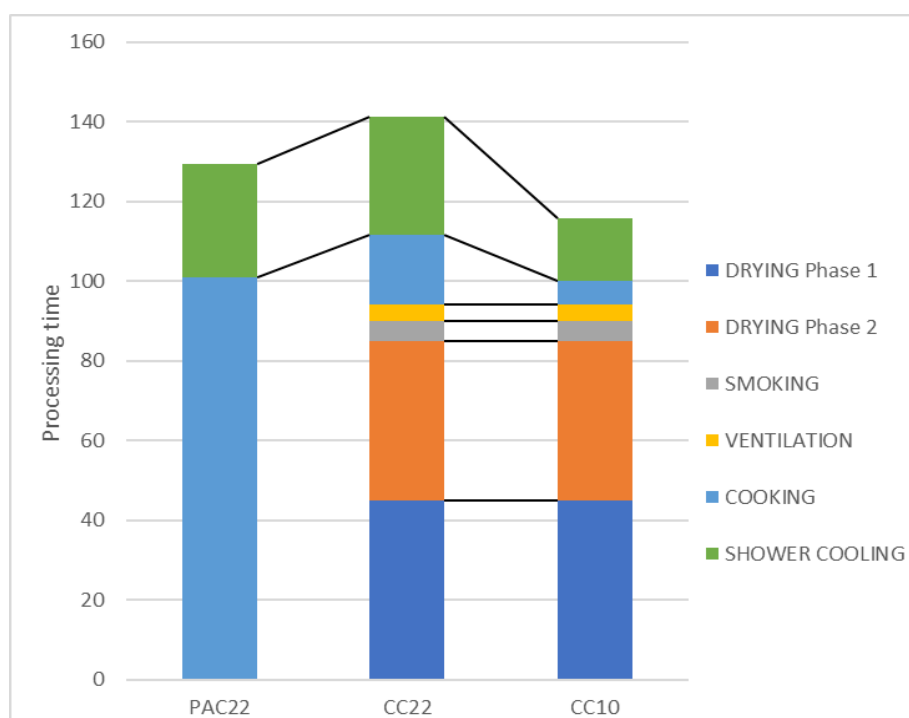


Figure 2. Total processing time

Two principal factors are reflected in the data: the type of casing material and the product diameter, both of which exert a demonstrable influence on the total processing time and on the distribution of time across individual phases (Barbut, 2024, Suurs & Barbut, 2020).

Both CC22 and CC10 were subjected to an identical drying, smoking, and ventilation phase before the cooking phase, each requiring 85 minutes for the combined drying phases and 5 minutes for smoking. PAC22, by contrast, underwent only the cooking phase. The selection of casing material is directly linked to the intended thermal treatment: cellulose casings are employed specifically because they permit the diffusion of smoke components and the evaporation of surface moisture, thereby facilitating more effective heat transfer into the product core (Suurs & Barbut, 2020). The penetration of smoke also contributes additional thermal energy, partially pre-heating the batter before the cooking phase commences, while the deposition of phenolic compounds simultaneously imparts antimicrobial and antioxidant protection (Łopusiewicz et al., 2021).

Despite the greater permeability and associated thermal advantages of the cellulose casing, the CC22 required a longer total processing time (141.3 min) than the PAC22 (129.3 min). This apparent contradiction is explained by the structure of the thermal regime: the polyamide-cased product was cooked at +85°C from the outset, whereas the cellulose-cased product underwent extended drying and smoking at lower temperatures (60–70°C) before cooking commenced. The net effect is that, although the cellulose casing offers superior heat transmission during the cooking phase, the lower temperatures sustained during the drying and smoking phases prolong the overall processing time (Rašeta et al., 2023; Živković, 1986).

The results of total process monitoring across all three product variants and three batches each are presented in Tables 2-4. For PAC22 samples, thermal processing time was 101 minutes, and total processing time 129.3 minutes. For CC22, total processing times was 141.3 minutes, of which the cooking phase 17.7 minutes. For CC10, total processing time was 116 minutes, with cooking time of 6 minutes.

As noted by Vuković (2012), strict adherence to defined time-temperature profiles during thermal processing is a prerequisite for the destruction of pathogenic and spoilage microorganisms and for achieving the required minimum core temperature of 72°C.

A comparison of Tables 2 and 3 reveals a marked difference in the duration of the cooking phase between the two Ø 22 mm product variants. Samples PAC22, without drying and smoking phase (Table 2), required a mean cooking time of 101.0 minutes to reach a core temperature of 72°C, while samples CC22, with a full drying, smoking and cooking phase (Table 3), required 111.7 minutes to reach the target core temperature. This apparent difference reflects the distinct heat transfer characteristics and the thermal pre-conditioning effect of the drying/smoking phase. During drying and smoking, the product surface and sub-surface layers undergo progressive heating, substantially reducing the additional cooking time required to achieve the target core temperature (Rašeta et al., 2023; Vuković, 2012).

Table 2. Processing parameters – PAC22

Batch (150 kg)	Cooking phase		Thermal processing time	Shower cooling phase	Total time
	T (°C)	t (min)	t (min)	t (min)	t (min)
Batch 1	85	101	101	29	130
Batch 2	85	104	104	28	132
Batch 3	85	98	98	28	126
Average		101.0	101.0	28.3	129.3

In contrast, the polyamide-cased product received no prior thermal input before the cooking phase, as polyamide is impermeable to smoke. These findings are consistent with the established relationship between casing permeability, heat transfer efficiency, and processing time described by Živković (1986).

The most pronounced influence on total thermal processing time was the product diameter. Samples CC10 (Table 4) completed the whole process with a mean total time of 115.7 minutes, compared to

141.3 minutes for samples CC22 under an identical smoking and cooking phase. The mean cooking time for CC10 was 6.0 minutes, compared to 17.7 minutes for CC22. This difference is a direct consequence of the inverse relationship between product diameter and heat penetration rate: smaller diameters present a shorter thermal path to the product center, and heat conduction therefore achieves the required core temperature more rapidly (Vuković, 2012).

Table 3. Processing parameters – CC22

	Drying phase				Smoking phase	Venting phase	Cooking phase	Thermal processing time	Shower cooling phase	Total time		
	1		2									
Batch (75 kg)	T (°C)	t (min)	T (°C)	t (min)	T (°C)	t (min)	t (min)	T (°C)	t (min)	t (min)	t (min)	t (min)
Batch 1	60	45	65	40	70	5	4	85	16	110	27	137
Batch 2	60	45	65	40	70	5	4	85	17	111	29	140
Batch 3	60	45	65	40	70	5	4	85	20	114	33	147
Average		45		40		5	4		17.7	111.7	29.6	141.3

These results confirm the principle that thermal processing regimes must be calibrated specifically to product diameter. As noted by Živković (1986), the heat treatment duration is determined not only by the processing temperature and the thermal properties of the batter, but critically by the product's cross-sectional diameter, which controls the rate of heat conduction through the meat matrix. This principle has been formally validated for frankfurter-type sausages of varying diameters in experimental work by Rašeta et al. (2025). This variability likely reflects minor differences in initial batter temperature, loading density within the processing chamber, and local airflow patterns, all of which influence heat transfer to individual products (Feyissa & Frosch, 2023).

Table 4. Processing parameters – CC10

	Drying phase				Smoking phase	Venting phase	Cooking phase	Thermal processing time	Shower cooling phase	Total time		
	1		2									
Batch (75 kg)	T (°C)	t (min)	T (°C)	t (min)	T (°C)	t (min)	t (min)	T (°C)	t (min)	t (min)	t (min)	t (min)
Batch 1	60	45	65	40	70	5	4	85	5	99	16	115
Batch 2	60	45	65	40	70	5	4	85	7	101	16	117
Batch 3	60	45	65	40	70	5	4	85	6	100	15	115
Average		45		40		5	4		6.0	100	15.7	115.7

The importance of reliable temperature measurement technology in this context represents the foundation of effective Critical Control Point management under HACCP principles (Vuković, 2012, Živković, 1986).

The thermal processing conditions applied in this study, cooking temperature of 85°C with core temperatures reaching 72°C, are consistent with the pasteurization approach standard for cooked sausage products. At these temperatures, vegetative cells of relevant pathogens, including *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*, are effectively inactivated, with a reduction of at least 6 log CFU/g achievable for *L. monocytogenes* at 72°C (Rašeta et al., 2023; Vuković, 2012). However, as noted in the literature, pasteurization temperatures are insufficient to destroy heat-resistant endospores of *Bacillus* and *Clostridium* species, which necessitates the maintenance of an unbroken cold chain at $\leq 4^{\circ}\text{C}$ throughout storage and distribution to prevent post-process germination and outgrowth (Munir et al., 2023; Živković, 1986).

The most pronounced effect of product diameter is observed in the cooking phase. For the CC10, a mean cooking time of only 6.0 minutes was sufficient to achieve a core temperature of 72°C, compared to 17.7 minutes for the CC22, equivalent composition but larger diameter. This difference is a direct consequence of the physical relationship between diameter and heat conduction: a smaller cross-sectional area reduces the distance over which heat must be conducted to reach the geometric center of the product, thereby accelerating the attainment of the critical minimum core temperature (Vuković, 2012). The total processing time of CC10, 115.7 minutes, was consequently the lowest among all three variants, despite sharing identical drying and smoking phases with the CC22, cellulose-cased product.

These findings underscore the need to establish product-specific thermal processing regimes that account for both casing permeability and product diameter. Applying a standardized, undifferentiated heat treatment to products of differing dimensions would inevitably result in either under-processing of larger-diameter products - with consequent food safety risk - or unnecessarily prolonged treatment of smaller-diameter products, adversely affecting product quality and energy efficiency (Rašeta et al., 2025). Precise, continuous temperature monitoring at the product core represents the only reliable method to verify that the critical control criterion of $\geq 72^{\circ}\text{C}$ is achieved across all product variants and positions within the processing chamber (Rašeta et al., 2023, Vuković, 2012, Živković, 1986).

CONCLUSION

This study confirmed that both casing material and product diameter exert a significant, measurable influence on the duration of individual thermal processing phases and on total processing time in the manufacture of finely comminuted cooked sausages. Cellulose casings, permeable to smoke and water vapor, enabled a combined drying–smoking–cooking phases and contributed to heat transfer efficiency through progressive surface pre-heating, whereas impermeable polyamide casings necessitated a cooking-only approach with a correspondingly longer cooking phase. Product diameter proved to be the most critical determinant of cooking duration: CC10 reached the required core temperature of 72°C after only 6.0 minutes, compared to 17.7 minutes for the CC22 variant of identical composition and casing type.

These findings highlight that product-specific thermal processing regimes, validated for each combination of casing type, product diameter, and processing sequence, are essential to ensure consistent safety and prevent under-processing associated with applying uniform heat treatment to products of differing dimensions. This confirms that both casing types and product diameter are critical determinants of thermal processing efficiency, underscoring the necessity of establishing and validating product-specific heat treatment regimes to ensure consistent safety in the manufacture of finely comminuted cooked sausages.

REFERENCES

- Barbut, S. (2024). Advancement in automation of co-extrusion collagen casings. *Frontiers in Soft Matter*, 4, 1356641. <https://doi.org/10.3389/frsfm.2024.1356641>
- Cheng, Q., & Sun, D. W. (2008). Factors affecting the water holding capacity of red meat products: A review of recent research advances. *Critical Reviews in Food Science and Nutrition*, 48(2), 137–159. <https://doi.org/10.1080/10408390601177660>
- Feyissa, A. H., & Frosch, S. (2023). An Integrated Model of Heat Transfer in Meat Products during Multistage Operations. *Foods*, 12(18), 3369. <https://doi.org/10.3390/foods12183369>
- Lee, S., Jo, K., Jeong, S. K., Jeon, H., Choi, Y. S., & Jung, S. (2023). Recent strategies for improving the quality of meat products. *Journal of Animal Science and Technology*, 65(5), 895–911. <https://doi.org/10.5187/jast.2023.e94>
- Łopusiewicz, Ł., Drożdowska, E., Sionek, B., Dąbrowski, R., Jaśkiewicz, K., & Tarczyńska, A. S. (2021). Effects of smoking temperature, smoking time, and type of wood sawdust on polycyclic aromatic hydrocarbon accumulation levels in directly smoked pork sausages. *Journal of Agricultural and Food Chemistry*, 69(3), 907–919. <https://doi.org/10.1021/acs.jafc.0c04116>

- Munir, M. T., Mtimet, N., Guillier, L., Meurens, F., Fravallo, P., Federighi, M., & Kooh, P. (2023). Physical Treatments to Control *Clostridium botulinum* Hazards in Food. *Foods* (Basel, Switzerland), 12(8), 1580. <https://doi.org/10.3390/foods12081580>
- Nizio, E., Czwartkowski, K., & Niedbała, G. (2023). Impact of smoking technology on the quality of food products: Absorption of polycyclic aromatic hydrocarbons (PAHs) by food products during smoking. *Sustainability*, 15(24), 16890. <https://doi.org/10.3390/su152416890>
- Rašeta, M., Mašić, Z., Pucarević, M., Vasin, A., Kos, I., Milošević, N., Plavša, N., & Teodorović, V. (2023). Ensuring the safety of cooked and smoked sausages of a narrower diameter, in a cellulose casing, by heat treatment. *Meat Technology*, 64(2), 133–143. <https://doi.org/10.18485/meattech.2023.64.2.87>
- Rašeta, M., Mašić, Z., Pucarević, M., Vasin, A., Kos, I., Milošević, N., Plavša, N., & Teodorović, V. (2025). Validation of pasteurization of finely chopped, cooked sausages with a small diameter. *Meat Technology*, 66(1). <https://doi.org/10.18485/meattech.2025.66.1.4>
- Sante-Lhoutellier, V., Astruc, T., Marinova, P., Greve, E., & Gatellier, P. (2008). Effect of meat cooking on physicochemical state and in vitro digestibility of myofibrillar proteins. *Journal of Agricultural and Food Chemistry*, 56(4), 1488–1494. <https://doi.org/10.1021/jf072999d>
- Savić, Z., & Savić, I. (2016). Sausage casings (2nd ed.). *Victus International*.
- Strzelczak, A., Balejko, J., Szymczak, M., & Witczak, A. (2021). Effect of Protein Denaturation Temperature on Rheological Properties of Baltic Herring (*Clupea harengus* membras) Muscle Tissue. *Foods* (Basel, Switzerland), 10(4), 829. <https://doi.org/10.3390/foods10040829>
- Suurs, P., & Barbut, S. (2020). Collagen use for co-extruded sausage casings – A review. *Trends in Food Science & Technology*, 102, 91–101. <https://doi.org/10.1016/j.tifs.2020.06.011>
- Vuković, I. (2012). Osnove tehnologije mesa (4th ed.). Veterinarska komora Srbije.
- Watson, S. C. (2025). Traditional meat preservation techniques and their modern applications. *Animal Frontiers*, vfaf051. <https://doi.org/10.1093/af/vfaf051>
- Zhang, G., Xu, G., Su, W., Zhang, Y., He, L., & Hou, Y. (2024). Effect of sterilization intensity on protein changes and structure in cooked sausage. *Meat Research*, 38(6), 27–33. <https://doi.org/10.7506/rlyj1001-8123-20240510-113>
- Zhu, N., Zhang, S., Zhao, B., Wang, X., Yin, J., & Zhou, G. (2020). Effect of processing on protein degradation and quality of emulsion sausages. *Food Bioscience*, 37(3), 100685. <https://doi.org/10.1016/j.fbio.2020.100685>
- Živković, J. (1986). Higijena i tehnologija mesa II dio – Meso u prometu i prerada mesa. Veterinarski fakultet Sveučilišta u Zagrebu / IRO Zadrugar.

Financial and strategic analysis of agricultural cooperatives in the Republic of Srpska: Transition from production to trade

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Abstract

In this paper, the financial and strategic sustainability of agricultural cooperatives in the Republic of Srpska was analyzed through a comparative analysis of two periods: 2014–2018 and 2022–2023. Based on financial reports from 54 active cooperatives, quantitative ratio analysis was combined with qualitative PESTLE and SWOT frameworks—derived from sector-wide strategic scanning—to identify a significant structural shift in the business model. Unlike the earlier period characterized by a focus on primary production, the 2022–2023 data reveal a sharp decline in revenue from the sale of own output (–40%) alongside a surge in revenue from the sale of goods (+67%), signaling a decisive pivot toward a trading orientation. This transition is accompanied by severe human capital erosion: the total workforce has been halved compared to the 2014–2018 average, with 21 cooperatives currently operating with zero employees. The study concludes that cooperatives are undergoing a structural transformation where trading has become the dominant strategy for maintaining liquidity and financial viability, albeit at the cost of long-term production capacity and their developmental role in rural areas.

Keywords: agricultural cooperatives, financial analysis, sustainability, structural transformation, the Republic of Srpska

INTRODUCTION

Agricultural cooperatives in the Republic of Srpska (RS) represent key actors in rural development, particularly for small and medium-sized producers. They enable joint production, market access, and better bargaining power, which is essential in conditions of fragmented and capital-weak agriculture (Bijman et al., 2012; Zelenika, 2018). Although strategic documents such as the Strategy for Agriculture Development of the RS (2019–2025) envisaged the improvement of institutional and financial support to the cooperative sector, empirical data show that the majority of cooperatives are failing to achieve planned goals and are facing stagnation.

Over the past decade, cooperatives in the RS have faced a series of challenges, highlighted by numerous sources (Cook, 1994; Chaddad & Cook, 2004; FAO, 2019), such as: market instability, price fluctuations of agricultural products, competition from industrial and imported goods, and a lack of labor force and capital. Weak internal organization and limited managerial capacity further hinder the ability of cooperatives to maintain a production focus and long-term sustainability (Bijman et al., 2012; Zelenika, 2018). These financial and operational vulnerabilities are particularly pronounced in the Western Balkans, where agricultural cooperatives struggle with severe capital constraints and require stronger institutional support to achieve financial sustainability (FAO, 2022).

This paper hypothesizes that external market pressures and a lack of internal capacities force agricultural cooperatives in the RS to transition from a production-based to a trade-based business model to ensure survival. This aligns with the broader concept of strategic adaptation, where cooperatives fundamentally alter their business models—such as shifting toward trade, consolidation, or diversification—to ensure longevity in turbulent external environments (Grashuis, 2018).

The aim of this research is to determine the actual financial and operational capacity of agricultural cooperatives in the Republic of Srpska to drive rural development through a comparative analysis of data for the periods 2014–2018 and 2022–2023. Special attention is paid to changes in revenue structure, human resources, and adaptive strategies of cooperative entities in the context of their sustainability and strategic transformation.

Numerous studies point to the importance of agricultural cooperatives in supporting small producers and maintaining rural development but also highlight their operational and financial challenges. Bijman et al. (2012) and Cook (1994) emphasize that cooperatives can increase market power and reduce transaction costs, but their success depends on internal capacities and managerial skills. In the context of Bosnia and Herzegovina and the Republic of Srpska, Zelenika (2018) highlights limitations in capital and human resources, while Pokharel et al. (2019) suggest that financial analysis of cooperatives provides key information for strategic decision-making. Similar findings are provided by local research (Stojanović & Stojanović, 2015; Vaško et al., 2016; the Ministry of Agriculture and Forestry of RS (2019–2025)), which shows that cooperatives often fail to achieve planned goals despite available institutional support.

This paper contributes to the literature by combining financial analysis with a strategic approach to the development of the cooperative sector in a transitional environment, building upon research that highlights the importance of internal capacities and network cooperation as key factors in the resilience of cooperative organizations (Cook, 1994; Chaddad & Cook, 2004; Bijman et al., 2012; Zelenika, 2018). The aforementioned studies support this paper's focus on the comparative analysis of the financial and operational performance of cooperatives across two periods as a basis for assessing their strategic transformation and sustainability.

MATERIAL AND METHODS

The research was based on secondary data derived from the financial reports of agricultural cooperatives registered in the Republic of Srpska. Data for the period 2022–2023 were obtained from the Agency for Intermediary, IT and Financial Services (APIF). The analysis included balance sheets, income statements, and accompanying notes to the financial statements of 54 cooperatives. While 54 cooperatives represent only a fraction of all registered entities, they constitute the entirety of the actively operating population that fulfilled the legal obligation of submitting annual financial reports to APIF for the observed years.

Data for the period 2014–2018 were sourced from relevant earlier research and official publications and were used for the purpose of comparative analysis of long-term trends. The comparative analysis encompassed two time intervals: the 2014–2018 period and the 2022–2023 period. The choice of these periods allowed for the assessment of long-term trends and the identification of changes in revenue structure, employment, and the financial position of cooperatives under varying macroeconomic conditions. To ensure comparability, the analysis was predominantly based on average values per cooperative and relative indicators (ratio analysis), thereby mitigating the impact of differences in the number of active cooperatives between the observed periods.

The analysis of financial and strategic sustainability was conducted using both quantitative and qualitative methods. Financial analysis was performed using ratio indicators grouped into four categories: liquidity (current ratio and quick ratio); activity (asset turnover ratio, receivables collection period, and inventory turnover period); financial structure (debt-to-asset ratio, long-term debt to equity ratio); and profitability (profit margin, return on assets – ROA, and return on equity – ROE). The indicators were calculated based on standard financial analysis formulas, and the results were interpreted in accordance with standard financial thresholds (e.g., current ratio ≥ 1) and the specificities of the cooperative business model.

Comparative analysis between the 2014–2018 and 2022–2023 periods was conducted using the descriptive method, comparing average and aggregated indicator values, with the aim of identifying structural changes in business operations. To assess the broader institutional and market environment, qualitative PESTLE and SWOT analyses were applied. The PESTLE analysis evaluated political, economic, socio-cultural, technological, legal, and environmental factors based on a systematic qualitative review of national strategic documents, sectoral reports, and secondary macroeconomic data, rather than primary empirical surveys. The findings from this environmental scanning were subsequently integrated into a SWOT matrix

to cross-reference the internal strengths and weaknesses of the cooperative sector with external opportunities and threats, providing a comprehensive framework for determining strategic sustainability. The integration of financial indicators and strategic analysis enabled a comprehensive assessment of the sustainability and transformation of the cooperative sector's business model.

The research results were interpreted with certain limitations, such as: the varying number of active cooperatives in the observed periods; changes in macroeconomic conditions (inflation, market fluctuations) that may affect nominal financial indicators; discontinuities in statistical series and trends and reliance on secondary data, without the possibility of detailed insight into the individual managerial decisions of cooperatives. Despite these limitations, the use of relative indicators and a comparative approach allows for the identification of reliable trends and structural changes within the cooperative sector.

RESULTS AND DISCUSSION

Structural Shift: Revenue Growth Alongside Production Decline

The analysis of trends in the financial indicators of agricultural cooperatives in the Republic of Srpska for the period 2022–2023 covered absolute values from balance sheets and income statements for a sample of 54 cooperatives. Considering the smaller number of cooperatives compared to the previously observed period (2014–2018), special attention was paid to average values per cooperative to obtain a realistic insight into the sector's financial and operational capacity. The analysis pointed to an interesting paradox: total sector revenues are rising, while production capacity and the number of employees are declining.

Table 1. Selected balance sheet, income statement, and structural items of cooperatives, 2022–2023 (in million BAM)

Income Statement, Balance Sheet, and Structural Items	2022	2023	Average (2022–2023)
Total revenues	26.5	28.6	27.6
<i>Revenue from the sale of goods</i>	<i>10.35</i>	<i>17.31</i>	<i>13.83</i>
<i>Revenue from the sale of own output</i>	<i>14.08</i>	<i>8.44</i>	<i>11.26</i>
Average revenue (per cooperative)	0.49	0.53	0.51
Total net profit	2.5	3.3	2.9
Profit before tax	2.7	3.5	3.1
Operating profit	3.1	3.5	3.3
Total assets	31.2	36.2	33.7
Total liabilities	16.2	19.2	17.7
Average liabilities (per cooperative)	0.30	0.36	0.33
Total equity	15.0	17.0	16.0
Number of loss-making cooperatives	9	16	12.5
Share of loss-making cooperatives (%)	16.7	29.6	23.2
Number of cooperatives with zero employees	19	21	20.0
Share of cooperatives with zero employees (%)	35.2	38.9	37.1

Source: Authors' calculation based on APIF data

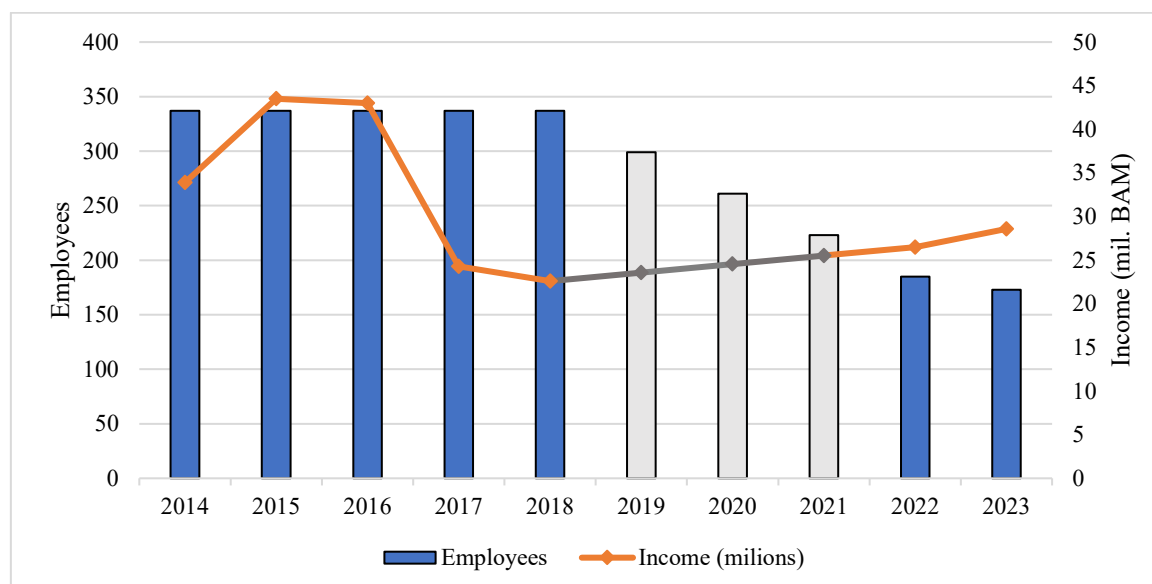
Total sector revenues increased by 8% (from 26.5 to 28.6 million BAM). However, it should be noted that this revenue growth is primarily nominal and largely driven by high inflation during the observed period, rather than an actual increase in production volume. While revenue from the sale of goods increased by 67%, revenue from the sale of own output recorded a decline of 40%. Total net profit increased from 2.5 to 3.3 million BAM, but simultaneously, the number of cooperatives operating with a loss increased (16 in 2023, representing 29.6% of the sample). It is particularly concerning that 38% of cooperatives have zero employees, while the average number of employees per cooperative dropped

from 4 workers in the 2014–2018 period to 3.2 workers in 2023. These data indicate that the positive aggregated financial result does not reflect a recovery of the sector as a whole, but rather a concentration of revenue in a smaller number of stronger entities.

Comparing these findings with the results of Stojanović and Rokvić Knežić (2021) for the 2014–2018 period, it is clear that a strategic shift in the business paradigm has occurred. Furthermore, the very fact that the number of active cooperatives submitting financial reports dropped from 83 to 54 additionally confirms the thesis of the sector's progressive erosion. While the production of own products dominated earlier, data for 2022–2023 indicate a conscious pivot toward a trading model, accompanied by resource optimization and more efficient business concentration.

The decline in the number of employees, from an average of 4 workers in the earlier period to a level where a significant number of cooperatives have no employees, can be interpreted as a result of rationalization and a transition to a model that requires less operational capacity but enables financial sustainability and increased profitability. The phenomenon of cooperatives operating with zero formal employees requires contextual clarification. This does not necessarily imply complete inactivity; rather, it reflects a structural adaptation where cooperatives function primarily as administrative or trading entities. As observed in sectoral analyses, operational tasks in such cooperatives are often outsourced or rely entirely on the temporary, seasonal engagement of cooperative members to coordinate purchasing and distribution. While this represents a reduction of fixed labor costs due to the seasonal nature of agriculture, reducing the formal workforce to zero highlights a severe lack of internal operational capacity and a decisive shift away from traditional, labor-intensive production models.

Compared to the 2014–2018 period, when the average revenue per cooperative amounted to 0.4 million BAM with a net loss of -1.4 million BAM, the 2022–2023 period shows improved profitability (average revenue 0.51 million BAM, profit 0.6 million BAM per cooperative), but also a higher concentration of risk and a smaller number of active cooperatives. These trends confirm the paper's hypothesis: a lack of internal capacities, combined with external market pressures, forces cooperatives to strategically transform into predominantly trade-oriented entities. Such a structural shift reflects a recognized phenomenon of strategic adaptation, where agricultural cooperatives prioritize short-term survival through commercial realignment when faced with severe resource constraints and an inability to independently finance modernization (Grashuis, 2018).



Graph 1. Trends in total revenues and number of employees in the RS cooperative sector (2014–2023): Revenue growth trend without employment growth. Note: Data for the transitional period (2019–2021) were not part of the primary APIF dataset analysis and represent a discontinuity in the statistical series.

Data source: Stojanović & Rokvić Knežić, 2021 for the 2014–2018 period; Stupar, 2026 for the 2022–2023 period

The combined graph illustrates the dynamics of income and employment in agricultural cooperatives in the Republic of Srpska over the periods 2014–2018 and 2022–2023, clearly pointing to a structural change in the sector. Data values for the years 2019, 2020, and 2021, for which no data were available, were obtained through linear interpolation between the 2018 and 2022 values. As stated by Stojanović and Rokvić Knežić (2021), 83 active cooperatives had a total of 337 employees. Total revenues varied: peaking in 2015 (43.5 million BAM) and dropping in 2018 (22.6 million BAM). In contrast, Stupar (2026), analyzing the 2022–2023 period, depicts a nominal recovery of total cooperative revenues from 26.5 million BAM (2022) to 28.6 million BAM (2023). However, the number of employees drastically decreased from an average of 337 workers in the 2014–2018 period to 173 workers in 2023, representing a decline of nearly 50%. While the absolute number of employees dropped partly due to the smaller sample of active cooperatives (54 vs. 83), productivity indicators per employee demonstrate actual economic growth. Specifically, revenue per employee increased significantly from the 2014–2018 period to 2023, largely because the remaining active cooperatives are handling higher trade volumes with a much smaller, core workforce.

This discontinuity between revenue trends and employment suggests productivity growth without a proportional increase in the workforce, but also points to potential processes of rationalization, job cuts, or changes in the organizational model of cooperatives. At the same time, this combination of mild financial recovery and significant workforce reduction confirms the paper's hypothesis: cooperatives are strategically shifting from a production model to a trade model, which requires less labor while ensuring the sector's financial sustainability. However, as shown in the graph, despite the revenue recovery, human resources are irreversibly declining, representing a long-term challenge for the competitiveness and sustainability of the cooperative sector in the Republic of Srpska.

Analysis of Financial Indicators

The comparative analysis of ratio indicators for agricultural cooperatives across two time periods enables an assessment of changes in the sector's financial position and business performance over time. Although the same analytical instruments were used in both cases (liquidity, activity, financial structure, and profitability ratios), it is important to emphasize that the analyzed periods differ in terms of macroeconomic conditions, the degree of market integration, and the institutional framework in which cooperatives operate.

Table 2. Key financial indicators of agricultural cooperatives in two time periods

Category	Indicator	2014–2018 (average)	2022	2023	Indication of Change
Liquidity	Current ratio	≈ 1.0	0.95	0.99	Slight improvement
	Quick ratio	n/a	0.58	0.64	Moderate growth
Activity	Receivables collection period (days)	n/a	45.7	56.7	Deterioration
	Inventory turnover period (days)	n/a	102.5	94.7	Improvement
Financial Structure	Debt-to-asset ratio	0.62	0.52	0.53	Growth in indebtedness
	Long-term debt / Equity	≈ 0.00	0.21	0.33	Investment cycle
Profitability	ROA	-0.01	0.08	0.08	Significant growth
	ROE	0.01	0.16	0.18	Continuous growth

Source: Authors' calculation based on Stojanović & Rokvić Knežić (2021) and Stupar (2026)

Table 2 presents a summarized comparative overview of key financial indicators for agricultural cooperatives in the two periods. Compared to the 2014–2018 period, cooperatives in 2022–2023 achieved a significant improvement in profitability indicators, alongside gradual growth in indebtedness and a slight improvement in liquidity. Although the efficiency of receivables collection deteriorated,

improved inventory management and a higher return on equity indicate structural changes and a more mature phase of development in the cooperative sector.

In the 2014–2018 period, the financial position of cooperatives was characterized by low profitability indicators, but relatively favorable solvency and moderate indebtedness. During that period, cooperatives showed a limited ability to generate profit, which was partly conditioned by their organizational nature and the dominant role of socio-economic goals over the profit motive. At the same time, the low use of long-term debt indicated a conservative financing model and the limited investment capacity of the sector.

In contrast, the analysis for the 2022–2023 period shows a gradual transformation of the cooperatives' financial profile. Although liquidity indicators remain below optimal values, a continuous growth trend is observable, indicating improved working capital management and better control of short-term liabilities. Unlike the earlier period, when liquidity was static and structurally limited, the newer period shows a visible trend of improvement, although still insufficient for long-term financial security.

A particularly significant difference between the observed periods relates to financial structure and indebtedness. While cooperatives in the 2014–2018 period used long-term financing sources to a very small extent, data for 2022–2023 indicate a more pronounced reliance on long-term loans. This change coincides with the decline in revenue from primary production and the simultaneous growth in revenue from trading activities, pointing to a certain transformation of financing sources. The increase in long-term liabilities can be linked to the initiation of an investment cycle aimed at modernization and capacity improvement, likely with the goal of stabilizing and diversifying revenue sources. Although such a policy resulted in a higher degree of indebtedness, the recorded improvement in net working capital indicates the preservation of short-term financial balance and the gradual strengthening of financial stability.

Regarding business efficiency, activity indicators in the 2022–2023 period show mixed trends. On the one hand, an improvement in inventory management was observed, while on the other hand, there was an extension of the receivables collection period and a decrease in asset turnover. Compared to the 2014–2018 period, this indicates a change in the structure of cooperative operations, which are increasingly facing market risks and demand limitations.

The most pronounced difference between the two periods is visible in profitability indicators. While very low or even negative rates of return were recorded in the earlier period, results for 2022–2023 show a stable level of ROA and growth in ROE, indicating increased capital efficiency. This confirms that, despite structural limitations, cooperatives have managed to improve economic performance and achieve a higher return on employed assets.

In summary, the comparative analysis shows that agricultural cooperatives in the Republic of Srpska in the 2022–2023 period achieved visible progress compared to the 2014–2018 period, particularly in terms of financial structure and profitability. However, challenges in the areas of liquidity and asset utilization efficiency remain present. These findings confirm that the further development of the cooperative sector is conditioned by the improvement of financial management, strengthening of the capital base, and continuous institutional support.

Personnel and Capital Vulnerability of Agricultural Cooperatives

The analysis of the cooperative sector in the Republic of Srpska in the 2022–2023 period reveals pronounced personnel and capital weaknesses. A concerning fact is that 21 out of a total of 54 cooperatives have zero employees, representing nearly 40% of the sample. An additional structural problem is the unfavorable age structure of the membership, with an average age approaching 60 years, indicating a lack of generational renewal and weak involvement of younger farmers (Stupar, 2026). Such a personnel structure significantly limits the ability of cooperatives to independently implement projects, manage production and administrative processes, and apply for EU funds, thereby directly threatening their long-term sustainability and development potential.

At the same time, management functions in a large number of cooperatives are performed by individuals without adequate professional managerial expertise, further complicating strategic planning, financial management, and adaptation to modern market conditions. The increase in credit exposure may indicate support for investment projects intended to enable the long-term strengthening of the sector's financial

sustainability and competitiveness. However, combined with the reduction in the number of employees and mildly rising revenues, it is clear that cooperatives today operate with significantly fewer human resources, pointing to a structural transformation of the sector and the need for additional institutional support and capacity-building programs.

External Environment of Agricultural Cooperatives

Stupar's (2026) analysis of the external environment of agricultural cooperatives in the Republic of Srpska points to complex challenges and opportunities for sector transformation. PESTLE analysis identified: unstable agrarian policies and legal gaps (P, L), inflation and high production costs (E), youth migration and workforce aging (S), obsolete equipment and slow digital development (T), as well as the pressure of climate change (E). At the same time, growing demand for domestic and organic products, as well as the availability of more modern technological solutions, represents a significant opportunity for cooperatives.

SWOT analysis highlights the key strengths and weaknesses of the sector. Cooperatives possess a developed membership network, local credibility, and institutional support, but are limited by financial capacities, a lack of personnel (primarily professional), and obsolete equipment. In this context, the WT (Weakness-Threat) strategy suggests unification and joint projects to reduce internal weaknesses and mitigate threats. The SO (Strength-Opportunity) strategy emphasizes using the sector's strengths to maximize market opportunities, including branding, digitalization, and focusing on trade margins. This approach confirms the paper's hypothesis: a lack of internal capacities and external market pressures force cooperatives to transform their business model from production to trade, rationalizing personnel and focusing on financial sustainability. The WT and SO strategies represent practical guidelines for survival and growth in the current environment (Stupar, 2026).

CONCLUSION

The comparative analysis of agricultural cooperative operations over the period 2014–2023 points to deep structural disruptions that call into question the effectiveness of previous rural development strategies. The research confirms the hypothesis that cooperatives, under the pressure of external and internal constraints, are strategically transforming from production-oriented to predominantly trade-oriented entities.

Comparing the results with Stojanović and Rokvić Knežić (2021), a disruption of the positive trend of growth in own production, which characterized the period up to 2018, is observed. In 2022/2023, despite a nominal growth in total revenues (+16%), the sector recorded a sharp decline in revenue from the sale of own output (−40%) and significant growth in revenue from the sale of goods (+67%). This dynamics suggests that cooperatives, lacking long-term capital and professional resources, are choosing the trading model as a less risky way to preserve liquidity.

The most alarming finding is the erosion of human capital. The average has fallen from 4 to 3.2 employees per cooperative, with the structure of this decline being particularly alarming, as 21 out of 54 entities currently operate with zero employees. The loss of a critical mass of professional staff limits the ability of cooperatives to implement complex production processes or utilize EU funds, regardless of the availability of financial support.

It is concluded that the financial recovery of the sector in 2023 is apparent, as it is based primarily on trade margins rather than on creating new value through production. These structural trends align with broader international observations, which indicate that agricultural cooperatives often resort to strategic adaptation—such as transitioning toward trade, consolidation, or diversification—when faced with severe capital constraints, demographic shifts, and external market volatility. Similar survival mechanisms have been noted across the Western Balkans. However, in the context of the Republic of Srpska, this forced transition toward a trading model threatens the cooperatives' fundamental developmental and production-oriented role in rural areas. For long-term sustainability, it is necessary to urgently redefine the support model: focus on the professionalization of management and the return of human capacity to cooperatives, and develop financial instruments that recognize the specifics of cooperative assets, enabling them to refocus on production activities.

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REFERENCES

- Bijman, J., Bos, B., & Meester, G. (2012). *Agricultural cooperatives in Europe: Governance and financial performance*. Wageningen Academic Publishers.
- Chaddad, F. R., & Cook, M. L. (2004). Understanding new cooperative models: An ownership-control rights typology. *Review of Agricultural Economics*, 26(3), 348–360. <https://doi.org/10.1111/j.1467-9353.2004.00168.x>
- Cook, M. L. (1994). *The role of cooperatives in rural communities*. Cooperative Information Report 41, USDA.
- FAO. (2019). *The state of food and agriculture 2019. Moving forward on food loss and waste reduction*. Food and Agriculture Organization of the United Nations.
- FAO. (2022). *Agricultural cooperatives and financial sustainability in the Western Balkans*. Rome: Food and Agriculture Organization of the United Nations.
- Grashuis, J. (2018). An Exploratory Study of Cooperative Survival: Strategic Adaptation to External Developments. *Sustainability*, 10(3), 652. <https://doi.org/10.3390/su10030652>
- Ministarstvo poljoprivrede, šumarstva i vodoprivrede. (2019). *Program razvoja poljoprivrednih zadruga Republike Srpske 2019–2025*. Vlada Republike Srpske.
- Pokharel, K. P., Regmi, M., Featherstone, A. M., & Archer, D. W. (2019). Examining the financial performance of agricultural cooperatives in the USA. *Agricultural Finance Review*, 79(2), 271-282. <https://doi.org/10.1108/AFR-11-2017-0103>
- Stojanović, T., & Rokvić Knežić, G. (2021). Financial analysis of agricultural cooperatives in the Republic of Srpska. *X International Symposium on Agricultural Sciences, 27-29, May, 2021, Trebinje, Bosnia and Herzegovina. AgroReS 2021 Book of Proceedings*, 220-23
- Stojanović, T., & Stojanović, S. (2015). Finansijska analiza položaja preduzeća iz poljoprivrednog sektora Republike Srpske. *Agroznanje*, 16(4), 549-557. <https://doi.org/10.7251/AGRSR1504549S>
- Stupar, B. (2026). *Pojave i promjene koje određuju unutrašnje i spoljašnje okruženje za razvoj poljoprivrednih zadruga* [Master rad, Univerzitet u Banjoj Luci, Poljoprivredni fakultet].
- Vaško, Ž., Ostojić, A., Rokvić, G., Drinić, Lj., Mrdalj, V., Figurek, A., Brković, D. (2016). *Poljoprivreda i ruralni razvoj u Republici Srpskoj do 2020. godine*. Banja Luka: University in Banja Luka, Faculty of Agriculture.
- Zelenika, M. (2018). *Organizacija i razvoj poljoprivrednih zadruga u BiH*. Sarajevo: Ekonomski fakultet.

State and development trends of organic plant production in the Republic of Serbia

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Abstract

This paper examines the state and development trends of organic plant production in the Republic of Serbia over the period 2015–2024, with the aim of assessing growth dynamics, structural changes, and future development prospects. The analysis is based on official statistical data and international databases, applying comparative and trend analysis, including the use of base indices, growth rates, and linear regression for projection purposes. The results indicate a continuous expansion of organic production, as total areas under the organic farming system increased by 80.1% during the observed period, rising from 15,298 ha to 27,549 ha, with an average annual growth rate of 6.8% and a peak reached in 2023 (29,002 ha). Crop production dominates the structure of organic plant production, accounting for 53%, followed by fruit production (41.9%), while vegetables and other crops have a considerably smaller share. A decline in areas under cereals (26.7%) and industrial crops (53%) was recorded, in contrast to the significant growth of fodder crops, fruits, and especially medicinal and aromatic plants, which increased by more than 400%. Despite these positive developments, the share of organic agricultural land in total utilized agricultural land remains low, reaching only 0.83% in 2024. Comparative analysis shows that Serbia significantly lags behind the European Union and neighboring countries, while projection results indicate that this share may reach only about 1.16% by 2030, remaining far below both the EU target and current levels observed in surrounding EU member states. The results suggest that, although organic production demonstrates stable growth and favorable structural changes, its current level remains insufficient. Further development requires not only stronger institutional support and more efficient policy measures, but also improved market conditions, more stable demand and price incentives, better access to inputs such as organic seeds and fertilizers, and enhanced knowledge transfer and advisory support for producers.

Keywords: organic plant production, Serbia, growth trends, rural development, sustainability

INTRODUCTION

The modern concept of sustainable development is based on harmonizing economic activities with the principles of environmental protection and rational management of natural resources. Sustainable development essentially implies economic growth that does not produce negative environmental consequences. Among all economic sectors, agriculture is particularly dependent on environmental conditions and natural resources (Lješević & Markičević, 2009). In this context, sustainable agricultural development involves the management and conservation of natural resources and the direction of technological progress toward meeting the needs of present and future generations. Such development preserves natural resources, does not endanger the environment, and is economically viable and socially acceptable (Roljević et al., 2009).

Within this framework, organic agriculture stands out as one of the key practical models for implementing sustainable development. The introduction of organic production is considered a fundamental prerequisite

for sustainable agricultural development. Based on environmental protection principles, organic agriculture provides high-quality and health-safe agricultural and food products. It represents a production model that ensures ecological balance, improves the social status of farmers, and generates significant economic benefits.

The growing awareness of consumers regarding healthy food and the preservation of natural resources further stimulates the development of this sector. In recent decades, organic agriculture has become a significant factor of sustainable development. The production and trade of organic products have shown a continuous increase in their share of the agri-food market, thereby strengthening the competitive position of agriculture. Organic food and beverages represent a rapidly growing market segment in the global food industry. Although organic farming systems generally achieve lower yields compared to conventional agriculture, they are more profitable and environmentally sustainable (Reganold & Wachter, 2016).

The steady expansion of the organic products market, along with the growth in areas and the number of organic farms and producers, indicates a constant upward trend in organic production. The market for organic products remains unsaturated, as demand exceeds supply, leading many countries to rely on imports. Increased awareness of healthy lifestyles has further intensified interest in organic products. Although production has grown significantly, it still lags behind demand, creating substantial opportunities for further sectoral development (Tabaković et al., 2017). The increasing consumer interest in organic products in Europe and Serbia, combined with high-quality agricultural land, limited supply on foreign markets, and higher prices compared to conventional products, makes organic agriculture an attractive field for investment (Jovanović et al., 2018).

The Republic of Serbia possesses considerable natural and market potential for the development of organic production. Large areas of rural land remain unpolluted by industrial waste or intensive conventional production. Small agricultural holdings in rural areas are increasingly adopting organic practices while simultaneously promoting non-conventional tourism forms such as rural tourism, ethno-tourism, and eco-tourism (Beslač et al., 2023). Favorable climatic conditions and high natural potential position Serbia among countries where organic production can be successfully developed, while organic products can contribute to national economic growth and market recognition.

Against this background, the paper aims to assess the current state and development trends of organic plant production in the Republic of Serbia. The analysis focuses on changes in the total area under organic production and its structural composition, including both certified areas and those in the conversion period. It also examines the share of organic plant production within total utilized agricultural land in order to evaluate its relative importance in the overall agricultural system. Furthermore, a comparative analysis of the share of organic agricultural land in the European Union and selected neighboring EU member states is included, providing a broader perspective on Serbia's position and development potential in this sector.

MATERIAL AND METHODS

The data used in this study were obtained from the official publications and database of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, as well as European and global organic farming statistics (FIBL Statistics). The database was constructed based on the areas under organic plant production, including areas in the conversion period, for the time span from 2015 to 2024. The selected period is determined by the availability and consistency of official statistical data, enabling a comparable analysis over a ten-year timeframe.

According to the Law on Organic Production ("Official Gazette of the Republic of Serbia", No. 30/10 and subsequent amendments) and the accompanying bylaws, areas under organic production are classified into areas with fully established organic status, which have completed the prescribed conversion period and obtained certification, and areas in conversion, which represent a transitional period (typically lasting two to three years) during which production is carried out in accordance with organic standards but without full certification status.

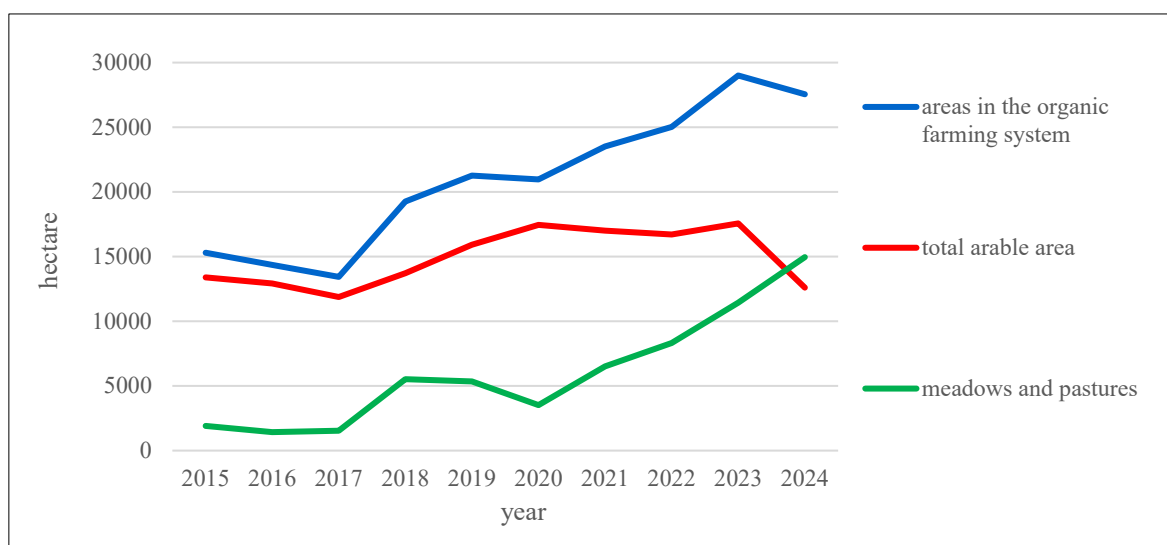
The methodological approach is based on comparative and trend analysis, using base indices and percentage changes. Trend projections were performed using linear regression analysis applied to time

series data. The base index presented in Table 1 is expressed relative to the base year (2015 = 100), allowing for the assessment of overall changes during the observed period.

RESULTS AND DISCUSSION

The Republic of Serbia has significant prospects for organic agricultural production due to available arable land, natural reserves, forest zones, and high climatic diversity. Favorable soil characteristics, climatic conditions, and water resources provide a substantial, though insufficiently utilized, potential for organic agriculture development.

During the previous decade, the average area under organic production in Serbia amounted to approximately 21,000 ha, with a continuous increase in total organic farmland (Graph 1).

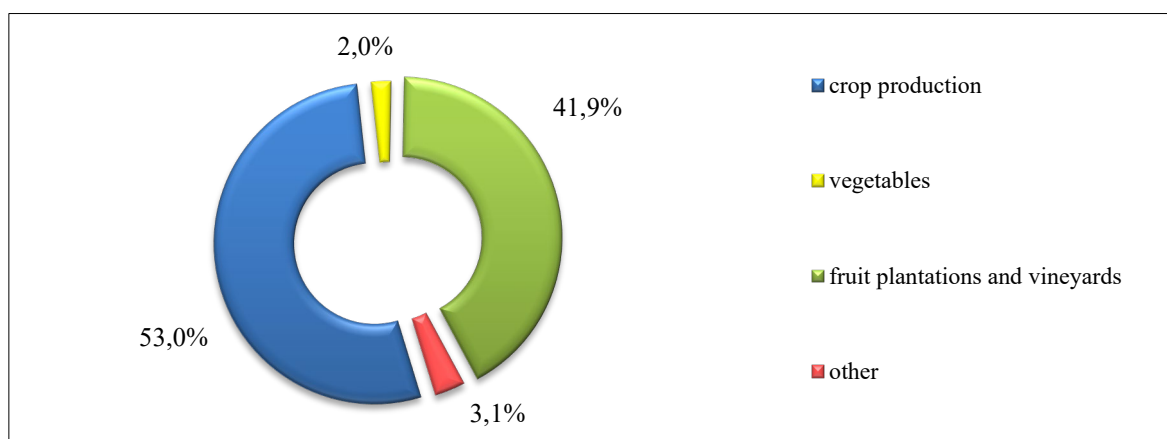


Graph 1. Areas under organic production in the period from 2015 to 2024

Source: Authors' presentation based on <https://minpolj.gov.rs/>

During the observed period, organic areas increased by 80%, with an average annual growth rate of 6.8%, reaching a maximum of 29,000 ha in 2023.

Regarding the structure of organic plant production, crop production accounts for 53% of total organic areas (6,672 ha in 2024), followed by fruit and grape production with 41.9% (5,279 ha), vegetable production with 2%, and other crops with 3.1% (Graph 2).

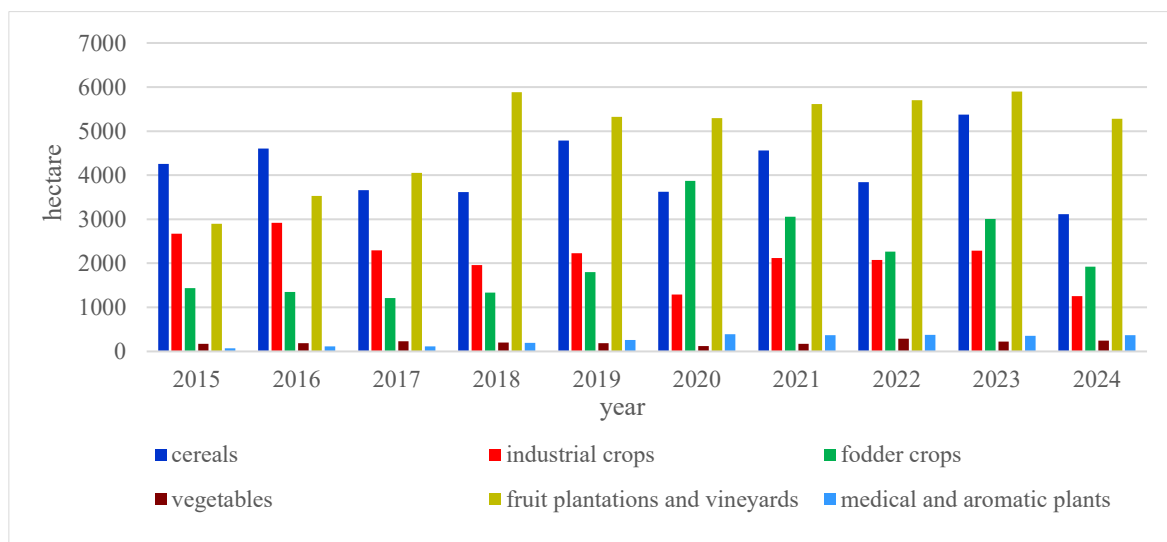


Graph 2. Structure of organic plant production in the period from 2015 to 2024

Source: Authors' presentation based on <https://minpolj.gov.rs/>

Within crop production, cereals dominate with a 46.7% share, followed by fodder crops (28.9%), industrial crops (18.8%), and medical and aromatic plants (5.6%). In fruit production, raspberry is the leading crop, covering 1,419 ha and accounting for nearly 27% of total organic fruit production.

Significant growth has been recorded in fodder crops, vegetables, fruits, and especially medical and aromatic plants (ranging from 33.7% in the case of fodder plants up to 424% in the case of medicinal and aromatic plants), while cereal production decreased by 26.7% and industrial crops by 53% (Graph 3).

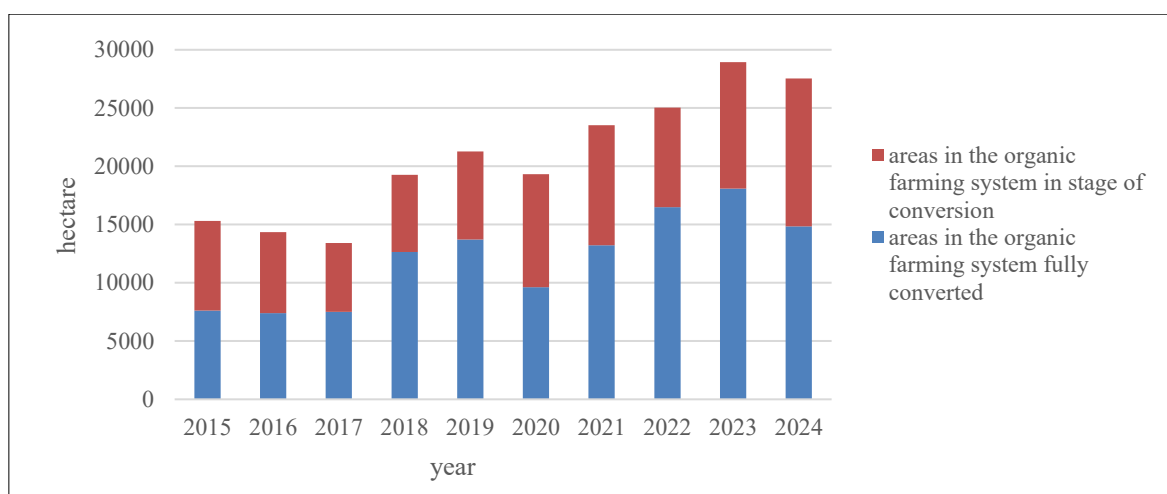


Graph 3. Structure of arable land by types of organic plant production in the period from 2015 to 2024

Source: Authors' presentation based on <https://minpolj.gov.rs/>

The decline in the area under cereals within the organic production system may negatively affect the overall volume of production in the forthcoming period. It may indicate that producers are dissatisfied with the level of income and therefore reduce the cultivated area. This may also be conditioned by an inadequate level of subsidies or an inappropriate credit policy in this domain. Furthermore, it may suggest the existence of certain production problems caused by agroecological conditions. One of the reasons may also be weak market demand or export difficulties, which may lead to a decline in production in the upcoming period (Vlahović et al., 2024).

In the observed period, an increase was recorded in the total area under organic production, including both areas with full organic status and areas in the conversion period (Graph 4).



Graph 4. Areas in the organic farming system according to status in the period from 2015 to 2024

Source: Authors' presentation based on <https://minpolj.gov.rs/>

Areas with full organic status amounted to 7,628 ha in the initial year of observation, while in the final year they reached 14,835 ha, representing an increase of 94.5%. Areas in the conversion period totaled 7,670 ha in the initial year and 12,705 ha in the final year, representing an increase of 65.6%. Greater fluctuations were recorded in areas with full organic status, which may be caused by a combination of factors. One factor is that the organic system often requires high costs per unit of area; therefore, producers faced with higher expenditures may decide to return to conventional production. If the market does not provide favorable conditions for the placement of organic products or if demand is weak, producers may assess that maintaining the same production volume is not economically justified. Dissatisfaction with achieved prices may lead to a reduction or complete abandonment of organic production. The reduction of areas with organic status is partly caused by the exclusion of certain parcels that no longer meet the prescribed conditions for organizing this type of production. Finally, declining interest in this type of production may be associated with insufficient institutional support. Mild oscillations in areas in the conversion period are primarily caused by the economic demotivation of producers during the process of fulfilling all prescribed requirements for the establishment, control, and certification of organic production, as well as by a lack of knowledge and information in this field.

Areas within the organic farming system, as well as their share in total utilized agricultural land, increased by 80.1% and 88.6%, respectively, during the observed period, with average annual growth rates of 6.8% and 7.3% (Table 1).

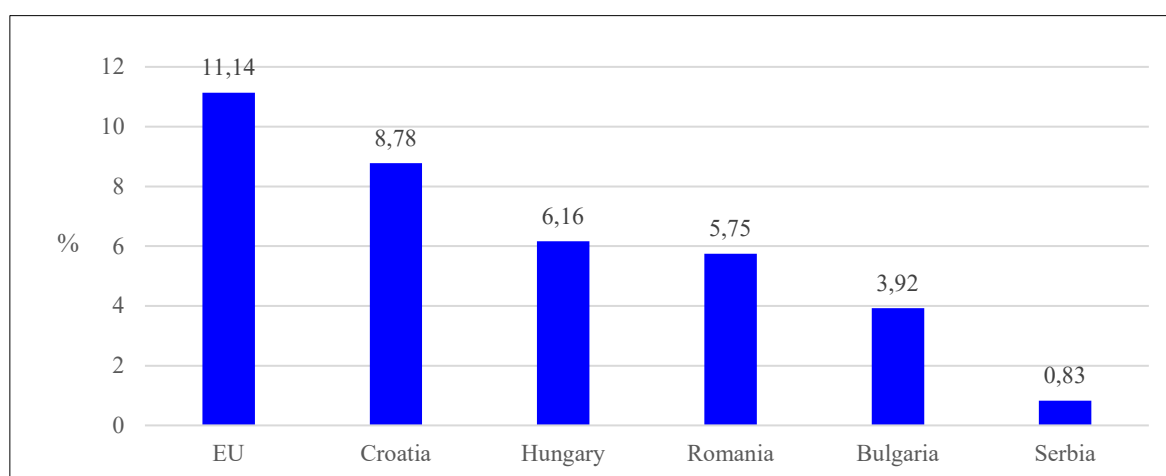
Table 1. Share of areas under organic plant production in total utilized agricultural land in Serbia in the period from 2015 to 2024

Year	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	Index
Areas in the organic farming system (ha)	15298	14358	13432	19254	21265	20970	23527	25035	29002	27549	180,1
Organic area share in total utilized agricultural land (%)	0,44	0,41	0,39	0,55	0,61	0,6	0,68	0,72	0,85	0,83	188,6

Source: Authors' calculation based on <https://minpolj.gov.rs/>

Despite the achieved growth, the share of areas within the organic farming system in total utilized agricultural land remains extremely low and has not reached 1%, with the share recorded in 2024 amounting to 0.83%. These results indicate that there is substantial potential for a more dynamic development of organic production.

To place Serbia's organic sector into a broader context, the share of organic agricultural land in total utilized agricultural area was compared with the European Union and some of neighboring countries, EU members (Graph 5).

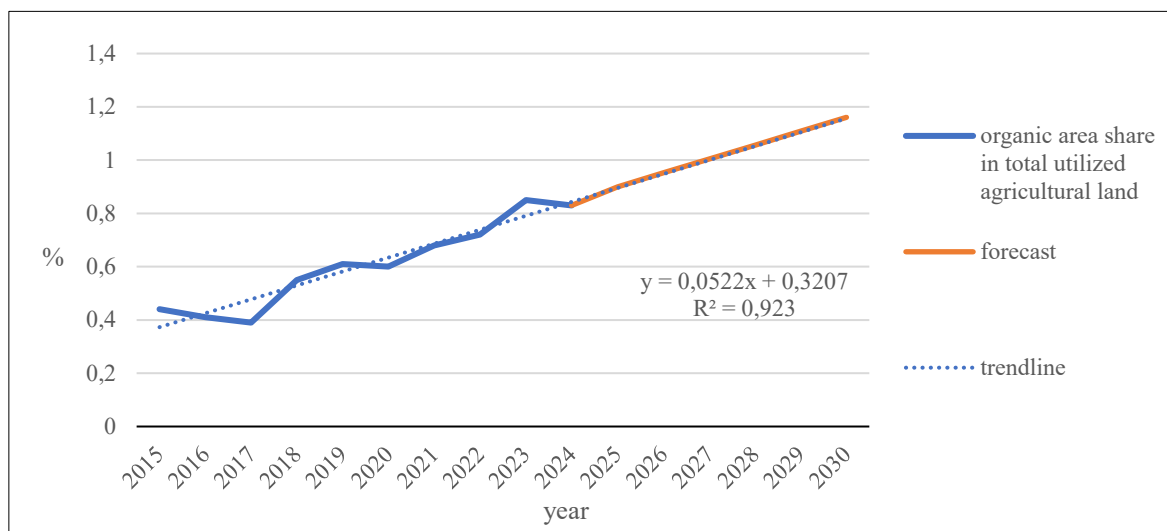


Graph 5. Organic area shares of total farmland in EU and selected countries in 2024

Source: Authors' presentation based on <https://statistics.fibl.org/>

The results indicate that Serbia significantly lags behind the EU level, where organic farming accounts for 11.14% of total agricultural land. Compared to neighboring countries, Serbia is below all observed EU member states according to organic area share of total farmland in the observed ten-year period, which ranges from 3.92% in Bulgaria to 8.78% in Croatia.

The growth observed in Serbia is quantitatively insufficient, despite a positive trend. Trend projections indicate that, if the share of organic area in total utilized agricultural land in Serbia continues to grow at the current average annual rate, it is expected to reach approximately 1.16% by 2030 (Graph 6).



Graph 6. Projected trend of the share of organic area in total utilized agricultural land in Serbia

Source: Authors' presentation based on <https://minpolj.gov.rs/>

These findings suggest that the current development trajectory is insufficient for convergence with EU standards. Even under a continuation of positive trend, Serbia would be substantially below current EU level, and far from reaching the EU target of 25% organic land by 2030 set in Farm to Fork Strategy. To achieve a 5% share of organic production in total utilized agricultural land by 2030, a significantly higher growth rate would be required (approximately 34–35% over a six-year period). This is substantially higher than the current average annual growth rate of 7.3%, indicating that achieving such a target would require a nearly fivefold acceleration of growth, supported by strong policy measures and structural changes in the agricultural sector.

The development of organic sector in Serbia can be strengthened through the implementation of a range of policy measures. One of the key elements is the financing of new technological solutions and the improvement of existing production methods, which can increase productivity, yield stability, and overall economic sustainability. Subsidies and financial support should facilitate producers' transition to the organic system and compensate for additional costs exceeding those of conventional production, accompanied by an appropriate system of tax incentives as an effective motivational instrument.

However, the sector faces numerous constraints. The biggest challenges faced by organic producers in Serbia are irregular and incomplete market supply with seed and planting material, especially in the required quantities; insufficient amounts of biological plant protection products, organic fertilizers, and compost (Nikolić et al., 2017). In addition, the sector faces a shortage of qualified personnel which is why educational and training programs are of particular importance.

As stated in the Strategy of Agriculture and Rural Development of the Republic of Serbia for the period 2025–2034 (Government of the Republic of Serbia, 2025), support should be provided for the development of bio-districts, defined as geographical areas in which farmers, citizens, tourism service providers, associations, and public institutions establish cooperative frameworks aimed at the sustainable management of local resources, based on the principles and practices of organic agriculture. Bio-districts also represent an effective instrument for encouraging producers within a given area to engage in organic production, while simultaneously facilitating market access for their products.

Given the limited capacity of domestic demand, institutional support for exports plays a particularly important role, enabling producers to access broader and more solvent markets, which in the long term contributes to the stability and sustainability of organic production.

Serbia possesses well-preserved natural resources suitable for the development of organic production. It is particularly significant that the largest share of agricultural production is carried out by small family farms, which, due to land fragmentation, have a comparative advantage in organizing organic production compared to conventional production.

Furthermore, the introduction and expansion of organic production stimulate the development of rural tourism through the creation of added value and increased recognition of local products. It also stimulates the employment of the working-age population, contributes to retaining young people in rural areas, strengthens the economic position of family farms, and encourages the inclusion of women in agribusiness, thereby influencing the reduction of unemployment and the revitalization of rural communities.

CONCLUSION

The research confirms that organic plant production in the Republic of Serbia has recorded a clear upward trend over the period 2015–2024, but at a pace that is still insufficient for more significant structural changes in the agricultural sector. Total areas within the organic farming system increased from 15,298 ha in 2015 to 27,549 ha in 2024, representing a growth of 80.1%, with an average annual growth rate of 6.8%. At the same time, the share of organic areas in total utilized agricultural land increased from 0.44% to 0.83%, which, despite an increase of 88.6%, remains at a very low level.

Structural analysis indicates that organic plant production in Serbia is dominated by crop production (53%), followed by fruit production (41.9%), while vegetables and other crops have marginal participation. Within crop production, cereals maintain the largest share (46.7%), although their area declined by 26.7%, alongside a significant reduction in industrial crops (53%). In contrast, strong expansion was recorded in fodder crops (33.7%), vegetables, fruits, and particularly medical and aromatic plants, which increased by as much as 424%. These changes suggest a gradual reorientation of producers toward more profitable and market-demanded crops.

Areas with full organic status increased by 94.5%, while areas in conversion grew by 65.6%, indicating continued interest in organic production despite noticeable fluctuations. These oscillations point to existing economic and institutional constraints, including high production costs, insufficient subsidies, market uncertainties, and limited access to inputs and knowledge.

Comparative analysis shows that Serbia significantly lags behind the European Union, where organic agriculture accounts for over 11% of total agricultural land, as well as neighboring EU countries, where this share ranges between 3.9% and 8.8%. The comparison between the current and required growth rates clearly illustrates the scale of the challenge. Under the current growth rate of 7.3%, the share of organic agriculture would reach only around 1.3% by 2030. In contrast, achieving a 5% share would require an average annual growth rate of approximately 35%, resulting in a steep exponential increase. The findings highlight that Serbia possesses substantial natural and economic potential for the development of organic production, but its utilization requires more intensive policy support. Key measures should include increased financial incentives, development of input markets, strengthening of advisory and educational systems, and support for export-oriented production. The development of bio-districts and integration with rural tourism could further contribute to sector expansion.

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REFERENCES

- Beslać, M., Cogoljević, V., & Vujović, S. (2023). Finansiranje organske proizvodnje i njen uticaj [Financing of organic production and its impact on the development of rural, ethno, and ecotourism in Serbia]. *Ecologica*, 30(111), 427-434. <https://doi.org/10.18485/ecologica.2023.30.111.12>
- European Commission (2020). Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52020DC0381>
- Government of the Republic of Serbia (2025). Strategy of Agriculture and Rural Development of the Republic of Serbia for the period 2025–2034 (draft). <https://www.agroklub.rs/dokumenti/nacrt-strategije-poljoprivrede-i-ruralnog-razvoja-za-period-od-2025-do-2034/7494/>
- Jovanović, O., Lazarević-Moravčević, M., & Đuričin, S. (2018). Organska poljoprivreda u funkciji održivog razvoja Republike Srbije [Organic agriculture in the function of sustainable development of the Republic of Serbia]. *Ecologica*, 25(91), 722-726.
- Law on Organic Production (2010). Official Gazette of the Republic of Serbia, 30/10 & 17/19. <https://serbiaorganica.info/wp-content/uploads/2021/03/Organic-production-in-Serbia-2020-ENG-online.pdf>
- Lješević, M., & Markićević, M. (2009). Geografske premise održivog razvoja poljoprivrede [Geographic premises of sustainable agricultural development]. *Glasnik srpskog geografskog društva*, 89(2), 127-134.
- Nikolić, S. R., Vuković, P., & Grujić, B. (2017). Measures to support the development of organic farming in the EU and Serbia. *Ekonomika poljoprivrede*, 64(1), 323-337.
- Reganold, J. P., & Wachter, J. M. (2016). Organic agriculture in the twenty-first century. *Nature Plants*, 2, 15221. <https://doi.org/10.1038/nplants.2015.221>
- Roljević, S., Hamović, V., & Sarić, R. (2009). Organska poljoprivreda u funkciji održivog razvoja [Organic agriculture in the function of sustainable development]. *Ekonomске teme*, 47(3), 99-109.
- Tabaković, M., Simić, M., Dragičević, V., & Brankov, M. (2017). Organska poljoprivreda u Srbiji. *Selekcija i semenarstvo*, 23(2), 45-53. <https://doi.org/10.5937/SelSem1702045T>
- Vlahović, B., Radojević, V., & Ivanić, I. (2024). Disperzija organske proizvodnje žitarica u Vojvodini [Dispersion of organic cereal production in Vojvodina]. *Agroekonomika*, 105, 29-44.

Production, trade and competitiveness of Serbia in the meat market

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Abstract

Negative trends observed in the livestock sector over the past decade highlight the need to examine the main developments in the production and trade of the key livestock products, as well as to analyse Serbia's position in regional and global markets. This paper examines trends in the production and foreign trade of meat and meat products over the five-year period (2020–2024), as well as assesses the competitive position of Serbia in the global meat market using selected trade and competitiveness indicators. The situation in the domestic market is analysed through the assessment of meat production (in total and by meat type), and using the self-sufficiency rate, which indicates the share of domestic demand satisfied by domestic production. Serbia's competitiveness in regional and global markets is analysed using export and import unit values, the relative unit value indicator, and two revealed comparative advantage indices. The results show that total meat production slightly increased during the observed period, but Serbia still does not hold a significant position, even at the European level. A self-sufficiency rate below 100% indicates Serbia's dependence on imports, primarily from European Union countries, to meet domestic demand for meat, which is also confirmed by the negative trade balance of this product group. Finally, the competitiveness analysis shows that Serbia achieves a limited competitive advantage only in the CEFTA market, and even this advantage has gradually weakened over the observed period, while its competitiveness in the European Union and global markets for meat and meat products remains low.

Keywords: meat and meat products market, foreign trade, competitiveness, Serbia

INTRODUCTION

Although Serbia has favourable natural conditions for the development of livestock production, this sector of agriculture has faced pronounced structural disruptions and long-term negative development trends over the past decades. Compared with the results of the Agricultural Census 2012, the data from the Agricultural Census 2023 indicate a significant decline in livestock production capacities. The number of agricultural holdings with livestock production activities decreased by 39%, while the total number of livestock units declined by 26%. The most pronounced decrease in the number of holdings occurred in cattle production (by 60%), while the largest reduction in the number of animals was recorded in pig production (by 34%). The decline in the total number of animals of almost all livestock species has also resulted in a decreasing contribution of livestock production to the total value of agricultural output in the country.

For this reason, it is particularly important to analyse how these structural changes in the livestock sector are reflected in the volume and structure of meat production, the level and dynamics of domestic consumption, as well as in the patterns of Serbia's foreign trade in meat.

Previous studies analysing meat production in Serbia predominantly point to a long-term declining trend that began in the early 1990s. Baltić et al. (2009) emphasize that, unlike the increasing trend of meat production in the world and the European Union, meat production in Serbia decreased from 626

thousand tons in 1991 to 474 thousand tons in 2007, with the most pronounced decline recorded in beef production. Similar conclusions are presented by Arsić et al. (2012), who identify an overall decrease in meat production in Serbia in the period 2000–2009, although with notable differences among meat types. While the production of sheep and poultry meat showed some growth, pork and beef production exhibited a negative trend. More recent studies point to certain changes in these dynamics. Branković Lazić et al. (2023) highlight a gradual stabilization and slight increase in total meat production after 2012. However, this growth has been accompanied by a significant increase in imports of meat and live animals, suggesting that the recovery of domestic production has not been entirely driven by stronger domestic production capacities, but by a growing reliance on foreign trade.

The modest and unstable level of meat production in Serbia has also affected its competitiveness on the international market. Serbia's export of meat during early XXI Century was predominantly directed toward regional markets, while competitiveness on the wider international market remained low. Mažić (2018) found that export prices of certain types of meat were significantly higher than the average world price as well as the prices achieved by leading exporters. Similar conclusions are presented by Branković Lazić et al. (2023), who note a slight increase in domestic meat production alongside a simultaneous rise in the value of imports of both meat and live animals, particularly pork. Additional insights into the sector are provided by the study of Mitić et al. (2018), which found that only around 10% of companies were export-oriented, indicating limited capacities to compete on international markets.

These developments point to a certain paradox in the development of the Serbian meat sector: despite signs of stabilization in domestic production, the increasing imports and changes in the structure of foreign trade indicate a growing dependence on international supply. In this context, the aim of this paper is to examine the recent developments in meat production, consumption and foreign trade in Serbia in the period 2020–2024, and to evaluate the country's level of self-sufficiency and its competitive position in the meat sector in regional and global markets, with particular reference to trade with the EU and CEFTA countries.

MATERIAL AND METHODS

For the purposes of this study, data from the Food and Agriculture Organization database (FAOstat) were used for the five-year period (2020–2024) and processed using Microsoft Excel. In the analysis of meat production and consumption, descriptive statistical measures were applied. The relationship between meat production and consumption was expressed through the self-sufficiency rate, calculated using the following formula:

$$SSR_{meat} = \frac{P_{meat}}{D_{meat}}$$

where SSR_{meat} represents the self-sufficiency rate in meat, P_{meat} is the total domestic meat production, and D_{meat} is total domestic consumption.

Foreign trade in meat was analyzed using the trade balance and coverage of imports by exports (import coverage ratio), while the competitiveness of Serbia on the international meat market was assessed using several indicators: export unit values (EUV), import unit values (IUV), the ratio of export to import unit values (Relative Unit Value – RUV), and the Revealed Comparative Advantage indicator (RCA).

Export unit value (EUV) is calculated as the ratio between the total value of exports and the exported quantity of a given product, while import unit value (IUV) is calculated as the ratio between the total value of imports and the imported quantity of a given product.

For a more detailed assessment of quality-based competitiveness, the Relative Unit Value (RUV) indicator is used, defined as the ratio between export and import unit values (EUV/IUV). RUV values in the interval from 0.85 to 1.15 indicate horizontal specialization, i.e., trade in products of approximately the same quality. Values below 0.85 indicate vertical specialization based on the export of lower-quality products and the import of higher-quality products, while values above 1.15 indicate vertical specialization based on the export of higher-quality products compared to imported products (Mrdalj et al., 2017; Božić et al., 2021).

In order to assess comparative advantages, the original Balassa index (RCA_1) and its modified form, the net export index (RCA_2), were calculated. RCA_1 is calculated as the ratio of the share of a specific product in a country's total exports to the share of that product in total world exports.

The original Balassa index is calculated using the following formula:

$$RCA_1 = \frac{\frac{X_{ij}}{X_{it}}}{\frac{X_{nj}}{X_{nt}}}$$

where X denotes exports, i represents the country, j the commodity sector, t the set of commodity sectors, and n the set of countries. Country i has a comparative advantage in the export of commodity sector (commodity group or product j), if $RCA_1 > 1$.

The original Balassa index includes only export, so an alternative indicator, RCA_2 , was also calculated using the following formula:

$$RCA_2 = \frac{(X_{ij} - M_{ij})}{(X_{ij} + M_{ij})}$$

where X denotes exports, M imports, i the country, and j the product.

RCA_2 represents the ratio between the foreign trade balance of a given country and the total trade volume of that country with the world. It is also referred to as the net export index (Božić & Nikolić, 2017). The value of the index ranges from -1 to 1. If RCA_2 takes values between 0 and +1, there is a revealed comparative advantage in that product (sector). If RCA_2 ranges from -1 to 0, the product (sector) does not have a comparative advantage.

In addition to the above indicators, the export potential of Serbian meat and meat products was assessed using the Export Potential Map database developed by the International Trade Centre (ITC). This tool provides estimates of unrealized export potential based on the comparison between current export flows and the level of demand in potential destination markets.

RESULTS AND DISCUSSION

Meat production

Total meat production in Serbia in the period 2020–2024 ranged from 517,017 t to 545,829 t, with an average annual growth rate of 1.36% (Table 1). The low coefficient of variation (2.04%) indicates a relatively stable level of total meat production during the observed period. Serbia is not a major meat producer on the global or even the European market, as its production accounts for only 0.15% of world production and 0.82% of European production (average for the period 2020–2024).

Table 1. Meat production in Serbia (average for 2020–2024)

Average production (t)	Range		CV (%)	Rate of change (%)	Share in production (%)	
	Min (t)	Max (t)			Global	Europe
528,472	517,017	545,829	2.04	1.36	0.15	0.82

Source: Authors' calculations based on the FAO Database, 2026.

The analysis of meat production by type shows that pork has the dominant share in the total meat production in Serbia, although with a slightly declining trend and a low degree of variability during the observed period. Chicken meat follows, with its share increasing from 22.1% in 2020 to 25.6% in 2024, indicating a gradual increase within the structure of meat production in Serbia.

These production trends are partly consistent with changes in consumption patterns. Average consumption of meat and meat products in Serbia reaches 67.3 kg per household member per year in the period 2021–2024, which is lower than the EU-27 average (79.1 kg), but significantly higher than the global average (43.8 kg). It should be noted, however, that the data for the EU and the world are based on FAO food

balance sheets, while the data for Serbia are derived from the Household Budget Survey, which should be taken into account when interpreting the results. Within the structure of meat consumption, pork traditionally occupies a dominant position; however, its consumption remained relatively stable during the observed period, fluctuating around 18 kg per household member annually. In contrast, the consumption of chicken meat increased from 17.5 kg to 20.5 kg per household member during the observed period. As a result, poultry consumption surpassed pork consumption in the last years of the observed period, indicating gradual changes in consumer preferences. This development is consistent with global trends, where poultry consumption has increased significantly, driven by economic, trade and dietary factors. Improvements in breeding efficiency and slaughter yields are also expected to support further growth in poultry production while partly mitigating the environmental impact of meat production (OECD-FAO Agricultural Outlook 2025–2034).

Poultry production in the EU is highly concentrated among several large producers, particularly Poland (20.5%), Spain (12.8%), France (12.2%), Germany (10.9%) and Italy (9.8%) (EC, 2025). Such a structure indicates a strong concentration of poultry production within the EU and suggests a relatively challenging competitive position for smaller producers, including Serbia, when accessing this market.

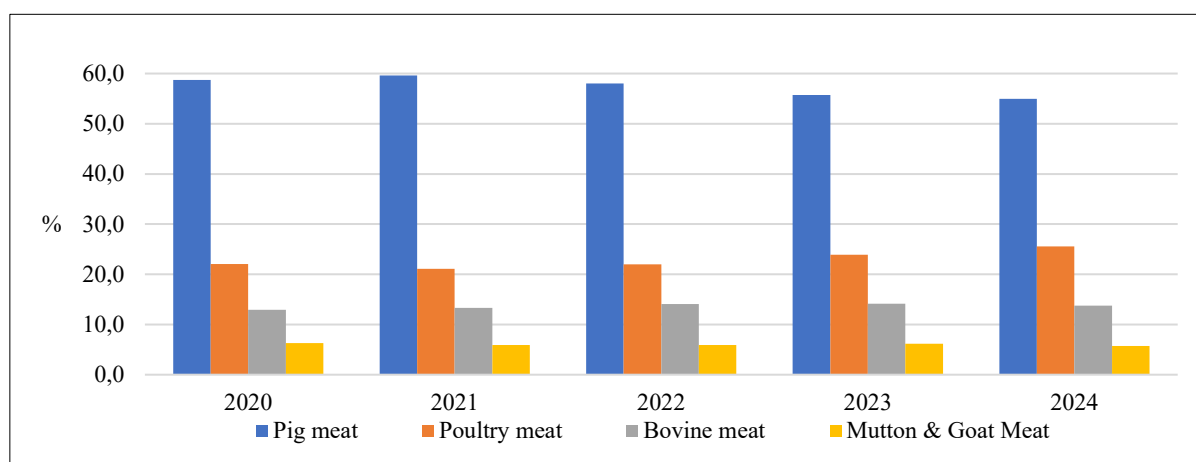


Figure 1. Structure of meat production in Serbia in the period 2020–2024

Source: Authors' calculations based on the FAO Database, 2026

Beef accounts for an average of 13.6% of total meat production, with only minor fluctuations during the analyzed years. Sheep and goat meat record the lowest shares in the structure of meat production, confirming their marginal but continuous presence in the overall meat production in Serbia. This is consistent to previous research, which also establishes the pattern of certain increase in sheep and goat meat production with decrease of other meat type production (Arsić et al., 2012). Such a trend may partly be explained by relatively low domestic demand, as the consumption of other types of meat (which is mostly sheep and goat meat) declined from 4.5 kg to 3.6 kg per household member.

These production patterns provide an important basis for understanding the level of meat self-sufficiency in Serbia and its position in international meat trade.

Self-sufficiency in meat production

In contrast to cereals, oilseeds and fruit, the level of meat self-sufficiency is the lowest among all groups of agricultural products and ranges from 93.8% to 89.2%, showing a declining trend (MAFWM, 2025), which represents an additional cause for concern. This trend reflects systemic problems in the livestock sector that have become particularly evident over the last decade and indicates the increasing import dependence of Serbia in the meat category.

However, differences in self-sufficiency levels can be observed across different types of meat. The production volume of beef and mutton indicates that domestic production is largely sufficient to meet the needs of the population for these types of meat. In contrast, a decline in self-sufficiency has been recorded in poultry and pork production (Table 2).

These trends may indicate the need to strengthen domestic livestock production as an important component of food sovereignty. At the same time, they also raise the question of the current utilization of the most important arable crops, whose surpluses are largely directed toward export, although they could potentially be used as feed resources to support the production of a larger number of livestock.

Table 2. Meat self-sufficiency rate in Serbia, 2020–2024 (%)

Year	Pig meat	Poultry meat	Bovine meat	Mutton & Goat meat	Total
2020	92.0	90.5	106.2	101.5	93.8
2021	90.0	91.3	104.2	99.6	92.5
2022	86.0	88.3	104.3	103.9	89.7
2023	86.1	87.9	100.0	102.3	89.2
Trend	↘	↘	↘	↗	↘

Source: Authors' calculations based on the FAO Database, 2026.

Foreign trade of meat and meat preparations

In order to better understand Serbia's position in the international meat market, the structure and dynamics of foreign trade in meat and meat preparations were analyzed by product categories and by major trading partners. This analysis provides an important basis for assessing the competitiveness of Serbia's meat sector.

In addition to trade in meat and meat preparations, Serbia also participates in international trade through exports of live animals. However, their role in total livestock exports has gradually declined. During the period 2020–2024, the export value of meat and meat preparations consistently exceeded that of live animals (Figure 2). While exports of meat and meat preparations remained relatively stable, exports of live animals showed a declining trend, particularly after 2021.

This pattern indicates a gradual shift away from exports of live animals toward products with a higher degree of processing. In general, exports of live animals are associated with lower value added, as most value creation occurs later in the production chain, often outside the exporting country. By contrast, exports of meat and meat preparations reflect a higher level of processing and allow for greater value capture within the domestic economy. Nevertheless, the continued presence of live animal exports suggests that Serbia still partly occupies a raw-material position within the livestock value chain, rather than a fully processing-oriented role.

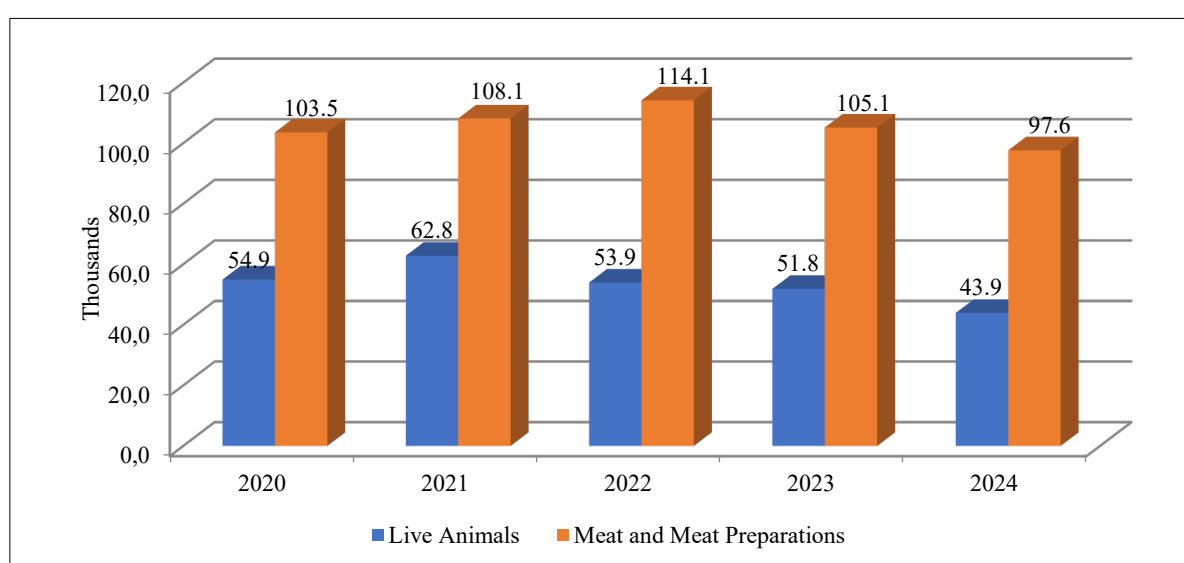


Figure 2. Exports of live animals and meat and meat preparations from Serbia (2020–2024)

Source: Authors' calculations based on the FAO Database, 2026.

The average annual value of exports of meat and meat preparations from the Republic of Serbia amounted to USD 105.7 million, while the average annual value of imports was significantly higher, reaching USD 260.3 million (Table 3). This indicates that Serbia is net importer of this group of products. The relatively stable level of export value, combined with the continuous increase in imports of meat and meat preparations, resulted in a growing trade deficit. By 2024, the trade deficit was approximately three times higher than in 2020. This trend is also reflected in the import coverage ratio, which declined from 57.4% in 2020 to 28.4% in 2024, indicating a deterioration in Serbia's trade position in this sector.

Table 3. Volume and dynamics of Serbia's foreign trade in meat and meat preparations, 2020–2024

	Average	Range of variation		CV (%)	Average annual growth rate (%)
		Min (t)	Max (t)		
Export (000 USD)	105,693	97,583	114,098	5.74	-1.47
Import (000 USD)	260,256	180,337	343,095	27.52	17.44
Trade balance (000 USD)	-154,563	-76,815	-245,513	-47.95	33.71
Coverage rate (%)	43.4	57.4	28.4	-	-

Source: Authors' calculations based on the FAO Database, 2026.

The structure of exports of meat and meat preparations shows that pig meat accounts for the largest share, averaging 34.6% of total exports during the period 2020–2024. Changes in the export structure were observed, reflected in the declining share of meat preparations, edible offal and beef, alongside a growing share of pig and poultry meat in total exports. In contrast, the structure of imports is strongly dominated by pig meat, which accounted for more than 65% of total imports in all observed years. Such a structure indicates a high dependence of the Serbian market on imported pork.

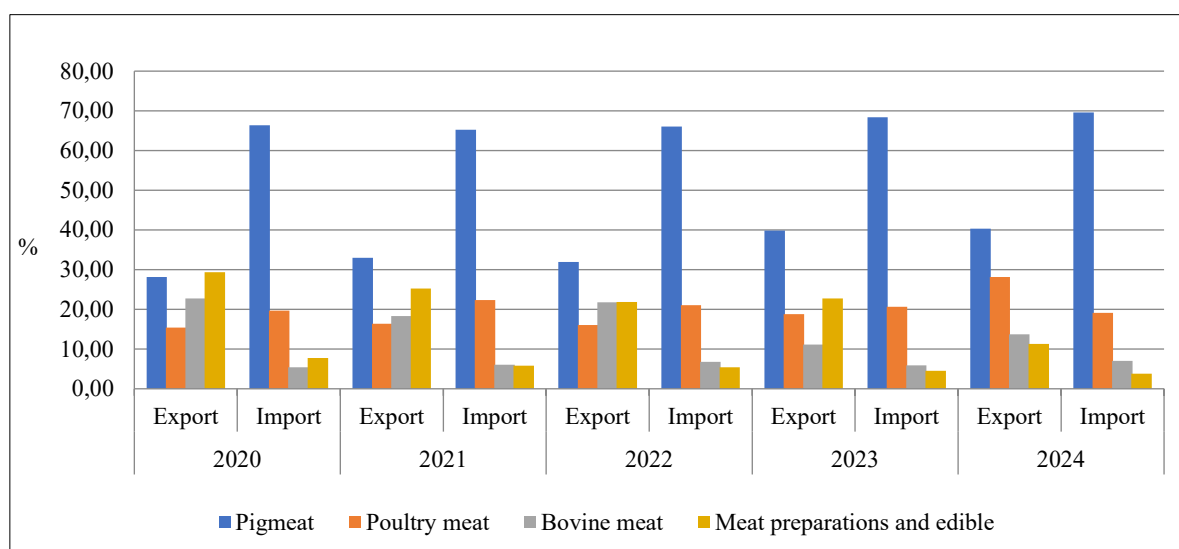


Figure 3. Structure of exports and imports of meat and meat preparations in Serbia, 2020–2024

Source: Authors' calculations based on the FAO Database, 2026.

The territorial distribution of exports shows a strong orientation toward CEFTA markets, which accounted for 82.2% of total export value in 2024 (Table 4). The EU represents the second most important export destination, while exports to other countries remain relatively limited. Among these markets, China stands out as the most important individual destination outside the regional market. On the other hand, the territorial structure of imports reveals a high dependence on the EU, although this dependence has shown a gradual decline in recent years. At the same time, imports from other countries have increased, with Russia emerging as an increasingly significant supplier.

Table 4. Territorial orientation of foreign trade in meat and meat preparations of Serbia, 2020–2024

		2020	2021	2022	2023	2024
000 USD	Export	103,522	108,131	114,098	105,131	97,583
	CEFTA	55.9	60.4	54.1	64.9	82.2
	EU-27	22.3	24.0	24.4	25.4	12.1
	Other countries	21.8	15.6	21.5	9.7	5.7
%	Import	180,337	196,569	264,479	316,798	343,095
	CEFTA	14.4	14.3	13.4	11.8	15.0
	EU-27	75.3	75.0	71.5	67.3	58.2
	Other countries	10.3	10.7	15.2	20.9	26.8
000 USD	Trade balance	-76,815	-88,438	-150,382	-211,667	-245,513
	CEFTA	31,855	37,084	26,405	31,008	28,789
	EU-27	-112,771	-121,407	-161,239	-186,575	-187,953
	Other countries	4,101	-4,115	-15,548	-56,100	-86,349

Source: Authors' calculations based on the FAO Database, 2026.

Trade balance indicators show that Serbia consistently recorded a trade deficit with the European Union in meat and meat preparations throughout the entire observed period. This deficit increased steadily and reached USD 187.9 million in 2024, the highest value in the analyzed period. Since 2021, Serbia has also recorded a negative trade balance in trade with other countries. These patterns of foreign trade indicate structural weaknesses in Serbia's meat sector, reflected in the strong dependence on imports, particularly from the EU, as well as the limited diversification of export markets. At the same time, the dominant role of regional CEFTA markets in Serbia's exports suggests that Serbian meat products face stronger competitive pressures on larger and more demanding international markets.

Competitiveness of Serbia on global meat market

During the period 2020–2024, the export unit value of meat and meat preparations from Serbia ranged between 2.88 and 4.08 USD per kilogram, while import unit values were slightly lower, ranging between 2.72 and 3.82 USD per kilogram. As a result, the relative unit value (RUV) remained above one throughout the observed period, with an average value of 1.10.

RUV values above one generally indicate that exported products achieve higher prices than imported ones, which may reflect either a higher level of product differentiation or the presence of niche market segments for higher-value products. Although RUV values above one may indicate the presence of higher-priced export products, this does not necessarily imply stronger competitiveness of the meat sector as a whole, particularly since there is a trade deficit. Basically, export unit values can be higher because small amounts of specific meat products are exported, but overall they do not generate enough income or provide competitive position of Serbia on global market.

RUV values above one generally indicate that exported products achieve higher prices than imported ones, which may reflect price-based positioning of exports rather than overall competitiveness. Such values may be associated with product differentiation or the presence of higher-value market segments. Although RUV values above one may indicate the presence of higher-priced export products, this does not necessarily imply stronger competitiveness of the meat sector as a whole. In this context, competitiveness should be assessed jointly with trade-based indicators, such as RCA and the trade balance. In the case of Serbia, despite RUV values above one, the presence of a trade deficit and RCA values below one indicates that higher export prices are not sufficient to ensure a competitive position in international markets. This may partly reflect the export of relatively small quantities of higher-value products, which does not translate into a strong overall export performance.

Table 5. Competitiveness indicators of meat and meat products in Serbia (2020–2024)

	EUV	IUV	RUV	RCA ₁			RCA ₂		
				World	EU	CEFTA	World	EU	CEFTA
2020	2.88	2.79	1.03	0.60	0.44	0.95	-0.27	-0.71	0.38
2021	3.12	2.72	1.15	0.54	0.39	0.91	-0.29	-0.70	0.40
2022	3.38	2.93	1.16	0.51	0.37	0.96	-0.40	-0.74	0.27
2023	4.01	3.70	1.08	0.43	0.30	0.84	-0.50	-0.78	0.29
2024	4.08	3.82	1.07	0.38	0.27	0.70	-0.56	-0.89	0.22
Average	3.49	3.19	1.10	-	-	-			

Source: FAO, 2026; Notes: EUV – Export Unit Values; IUV – Import Unit Values; RUV – Relative Unit Value (the ratio of export to import unit values); RCA – the Revealed Comparative Advantage.

The revealed comparative advantage index (RCA₁) confirms the relatively weak competitive position of Serbia in the international meat market. In all observed years, RCA₁ values remained below one in trade with the world, the EU and CEFTA countries (Table 5), indicating the absence of comparative advantages in meat exports. Moreover, the declining values of RCA₁ over the analyzed period suggest a gradual weakening of Serbia's export position.

A similar conclusion emerges from the net export index (RCA₂). Negative values of RCA₂ in trade with the world and the EU indicate a persistent trade deficit in meat and meat preparations. In contrast, positive RCA₂ values recorded in trade with CEFTA countries suggest the presence of a limited competitive advantage, although this advantage weakened in the past couple of years. It may be concluded that Serbia is regionally orientated exporter of meat and preparations (a positive trade balance with CEFTA) with some competitive position, but is gradually losing part of that position.

Given the dominant role of pork in Serbia's meat production and consumption structure, a separate analysis of competitiveness indicators was conducted for this meat category (Table 6).

Table 6. Competitiveness indicators of pork in Serbia (2020–2024)

	UVE	UVI	RUV	RCA ₁			RCA ₂		
				World	EU	CEFTA	World	EU	CEFTA
2020	3.63	3.06	1.19	0.58	0.27	1.00	-0.61	-0.97	0.37
2021	4.09	2.75	1.49	0.67	0.31	1.04	-0.56	-0.95	0.44
2022	4.26	2.92	1.46	0.72	0.32	1.00	-0.65	-0.96	0.33
2023	5.22	3.84	1.36	0.69	0.31	0.93	-0.68	-0.96	0.28
2024	5.43	3.83	1.42	0.63	0.29	0.76	-0.72	-0.99	0.13
Average	4.53	3.28	1.38	-	-	-	-	-	-

Source: FAO, 2026; Notes: EUV – Export Unit Values; IUV – Import Unit Values; RUV – Relative Unit Value (the ratio of export to import unit values); RCA – the Revealed Comparative Advantage.

During the period 2020–2024, the export unit value of pork ranged between 3.63 and 5.43 USD per kg, remaining consistently higher than the corresponding import unit values. Consequently, the relative unit value (RUV) for pork remained significantly above one throughout the observed period, with an average value of 1.38. This suggests that Serbian pork exports are positioned in market segments with relatively higher prices compared with imported pork.

However, RCA₁ values indicate that Serbia does not possess a strong competitive position in pork trade at the global and EU level. RCA₁ values remained below one throughout the observed period, pointing to the absence of comparative advantages in these markets. The RCA₂ index further confirms this pattern, while positive values of this indicator in trade with CEFTA countries suggest the existence of a certain level of competitive advantage, although this advantage declined toward the end of the analyzed period.

The competitiveness indicators for total meat and pork show broadly similar patterns. In both cases, RUV values exceed 1, while RCA_1 values remain below one and RCA_2 values are negative in trade with the world and the EU. This indicates higher export prices but the absence of strong comparative advantages in international markets. Positive RCA_2 values in trade with CEFTA countries suggest that Serbia's competitiveness in the meat sector and in the pork meat rest mainly on regional markets.

The establish pattern of Serbia's position on international meat market is confirmed by ITC Export Potential Map indices. Serbia still has a considerable unrealized export potential in the sector of meat and meat products, particularly in processed meat categories. The largest additional export potential is identified for processed meat products and sausages, whereas the potential for increasing exports of fresh meat appears to be significantly more limited (ITC, 2026).

A comparison between current export flows and estimated export potential further suggests that Serbia already exports certain products for which the remaining market potential is relatively small, while the largest unrealized opportunities are concentrated in higher value-added meat products. This indicates a certain mismatch between the current export structure and the structure of potential demand in international markets. These findings imply that future export growth may depend less on expanding exports of primary meat products and more on strengthening the processing segment of the meat sector. This also suggests that a more detailed analysis of export potential by product categories could provide additional insights into the future development of Serbia's meat exports.

CONCLUSION

The meat market in Serbia is characterized by numerous difficulties that affect both production performance and the country's position in the international market. Meat production in Serbia amounts to around 528 thousand tons and recorded modest growth in the period 2020–2024. Pork has the dominant share in total meat production in Serbia, which is also reflected in consumption patterns. However, poultry consumption surpassed pork consumption in the last years of the observed period, indicating gradual changes in consumer preferences.

The self-sufficiency rate is below 100% and declining, indicating a high dependence on imports to satisfy domestic demand. Exports of live animals have been decreasing, while the structure of exports has shifted toward meat and meat products. However, Serbia's trade balance shows a worrying trend of rapid deterioration. This is the result of a relatively stable level of export value combined with a continuous increase in imports of meat and meat preparations. As a consequence, the trade deficit in 2024 was approximately three times higher than in 2020. The structure of exports shows a strong orientation toward CEFTA markets, while imports of meat and meat products originate predominantly from the European Union.

Competitiveness indicators further confirm this pattern. Despite higher export unit values compared with import prices, Serbia does not exhibit revealed comparative advantages in trade with the European Union or in the global market. Limited competitive advantages are observed only in trade with CEFTA countries, although these advantages have weakened over the observed period.

Overall, the findings suggest that Serbia's position in the international meat market remains relatively weak. Strengthening production capacities, improving processing efficiency and enhancing export competitiveness represent key challenges for the future development of the Serbian meat sector.

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REFERENCES

- Arsić, S., Kljajić, N., & Vuković, P. (2012). Cattle stock and the analysis of total meat production in the Republic of Serbia. *Economics of Agriculture*, 59(1), 99–114. <https://doi.org/10.22004/ag.econ.123962>
- Baltić, M. Ž., Jovanović, S., Dimitrijević, M., Popović, L., & Mirilović, M. (2009). Meso svinja – proizvodnja i potrošnja [Pork meat – production and consumption]. *Veterinarski žurnal Republike Srpske*, 9(1), 4–10. <https://vet-erinar.vet.bg.ac.rs/handle/123456789/2329>
- Božić, D., & Nikolić, M. (2017). Spoljnotrgovinska razmena Srbije i značaj agrarnog sektora [Serbian foreign trade and importance of agrarian sector]. *Teme*, 41(3), 711–730. <https://doi.org/10.22190/TEME1703711B>
- Božić, I., Nikolić, M., & Božić, D. (2021). Značaj i konkurentnost kukuruza u spoljnoj trgovini Srbije i susednih zemalja [Significance and competitiveness of maize in foreign trade of Serbia and neighbouring countries]. *Agroekonomika*, 92, 1–13.
- Branković Lazić, I., Baltić, M., Simunović, S., Čirić, J., Baltić, T., Jovanović, J., & Đorđević, V. (2023). Examination of the volume of meat production and the value of meat imports to Serbia from 2012 to 2021. *Meat Technology*, 64(2), 500–504. <https://doi.org/10.18485/meattech.2023.64.2.96>
- European Commission. (2025). *Agricultural production – livestock and meat*.
- International Trade Centre (ITC). (2026). Export Potential Map. <https://exportpotential.intracen.org>
- Mažić, M. (2018). *Mogućnosti poboljšanja konkurentne pozicije Republike Srbije na međunarodnom tržištu mesa* [Opportunities for improving the competitive position of the Republic of Serbia in international meat market] [Doctoral dissertation]. University of Novi Sad, Faculty of Agriculture.
- Mitić, V., Kilibarda, N., Brdar, I., Kostić, M., Šarčević, D., Karabasil, N., & Mizdraković, V. (2018). Measuring competitiveness in the meat industry market: Are there any oligopolies in Serbia?. *Meat Technology*, 59(2), 127–136. <https://doi.org/10.18485/meattech.2018.59.2.8>
- Ministry of Agriculture, Forestry and Water Management. (2025). *Draft Strategy for Agriculture and Rural Development of the Republic of Serbia for the period 2025-2034*.
- Mrdalj, V., Ostojić, A., Vaško, Ž., & Brković, D. (2017). Structure and determinants of intra-industry trade in agro-food sector of Bosnia and Herzegovina. *European Journal of Interdisciplinary Studies*, 3(3), 154–163. <https://doi.org/10.26417/ejis.v3i3.154-163>
- Organisation for Economic Co-operation and Development & Food and Agricultural Organization of the United Nations. (2025). *OECD-FAO Agricultural Outlook 2025–2034*. <https://doi.org/10.1787/601276cd-en>

Consumer behavior towards local food products

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Abstract

This study examines consumer behaviour related to the purchase of local food products in the Banja Luka area, with a focus on the role of socio-demographic characteristics, purchasing power, and key attitudinal factors such as trust, perceived quality, and support for local producers. The research is based on primary data collected through a structured questionnaire using the CAWI method, with a sample of 150 respondents. Descriptive statistics, analysis of variance, Pearson correlation, and hierarchical cluster analysis were applied. The results indicate that local food products are characterized by a high level of consumer trust, with the majority of respondents perceiving them as higher quality compared to imported alternatives. Supporting local producers and trust in producers emerge as the most important factors influencing purchasing decisions, while price is of relatively lower importance. Statistically significant differences were observed across age and income groups, with older and higher-income respondents showing stronger preferences for local products. The analysis also reveals a strong positive relationship between purchasing power and food purchasing behaviour, highlighting the importance of economic capacity as a key determinant of actual consumption. Cluster analysis identified three distinct product groups, with traditional and minimally processed products showing the highest levels of consumer trust and loyalty, while processed products are less associated with local origin. The findings suggest that, despite favourable consumer attitudes, the development of local food markets in Bosnia and Herzegovina depends on improving the economic accessibility of local products and strengthening direct links between producers and consumers.

Keywords: local food products, consumer behaviour, consumer trust, hierarchial clustering

INTRODUCTION

In recent years, local food products have gained increasing importance in agri-food markets, driven by growing consumer interest in product quality, origin and trust in producers. At the same time, consumer behaviour in food markets has become more complex, reflecting the combined influence of socio-demographic characteristics, economic constraints and attitudinal factors. Local food systems are often associated with shorter supply chains, stronger links between producers and consumers and the preservation of traditional production practices. As such, they are increasingly recognized as an important component of rural development and local economic resilience. However, consumer preferences for local food are not uniform and depend on a range of factors, including income, age and perceptions of quality and reliability. Local food products are generally defined as goods originating from a specific geographic area, often produced using local resources and distributed through short supply chains (Rizvić & Agić, 2022). Beyond their geographic origin, such products are commonly associated with traditional production practices, authenticity and a closer relationship between producers and consumers (Janssen & Jager, 2002; Zarić et al., 2016). A substantial body of literature highlights that consumer preferences for local food are shaped by a combination of economic, demographic and attitudinal factors. Among these, consumer ethnocentrism plays a particularly important role, reflecting the tendency to favour domestic products over

imported alternatives (Shimp & Sharma, 1987; Matic, 2013). Empirical studies indicate that stronger ethnocentric attitudes are often associated with older consumers, rural populations, and individuals with a stronger sense of national identity, while younger and higher-income consumers may be more inclined toward imported products (Marinković et al., 2011; Akbarov, 2022; Candan et al., 2008). In addition to socio-demographic characteristics, purchasing decisions are strongly influenced by perceived product attributes, including quality, trust in producers, price, and country of origin (Kavak & Gumusluoglu, 2007). However, the relative importance of these factors varies across countries and market contexts. While some studies suggest that price sensitivity dominates in lower-income settings, others highlight the growing importance of quality and trust as key drivers of demand for local food products.

In the context of Bosnia and Herzegovina, consumer behaviour reflects a specific combination of structural constraints and attitudinal factors. The agri-food sector is characterized by relatively low domestic production capacity and a high dependence on imports, which significantly shapes the availability and competitiveness of local products on the market. At the same time, domestic production is often fragmented and based on small-scale farms, with limited integration into modern supply chains. Despite these structural limitations, consumers in Bosnia and Herzegovina demonstrate a relatively high level of trust in domestic food products and frequently associate them with higher quality, safety and authenticity. The purchase of local products is often perceived not only as an economic decision but also as a form of support for domestic producers and the national economy (Kasumović & Mehiljić Kalajdžić, 2013; Čivić & Čilimković, 2017). However, consumer choices are simultaneously constrained by purchasing power and price sensitivity, particularly in a context of lower average incomes and high exposure to imported products. This creates a dual consumption pattern in which positive attitudes toward local food do not always translate into actual purchasing behaviour. Recent evidence confirms that affordability and product availability remain key determinants of food purchasing decisions in Bosnia and Herzegovina (Gligorović, 2024).

Taken together, these findings suggest that consumer behaviour in Bosnia and Herzegovina is shaped by a complex interaction between structural market conditions and individual preferences. Therefore, the aim of this study is to analyse consumer behaviour related to the purchase of local food products in the Banja Luka area, with a particular focus on the role of socio-economic characteristics and key attitudinal factors such as trust, perceived quality and support for local producers. In addition, the study aims to identify differences in consumer preferences across demographic groups and to examine the relationship between purchasing power and food consumption patterns.

MATERIALS AND METHODS

The research is based on both primary and secondary data sources. Primary data were collected through a structured questionnaire using the CAWI method (Computer Assisted Web Interviewing). The survey was conducted in the area of Banja Luka on a sample of 150 respondents. Initially, 172 responses were collected, however after data validation 22 questionnaires were excluded due to logical inconsistencies, resulting in a final sample of 150 valid observations. The questionnaire consisted of two main sections. The first section included socio-demographic characteristics of respondents, such as age and income level. The second section focused on consumer attitudes and behaviour related to the purchase of local food products. Attitudinal variables were measured using a Likert scale, allowing respondents to express their level of agreement with predefined statements, while additional questions were structured in the form of closed-ended response categories. The collected data were coded and processed using Microsoft Excel and analysed in SPSS Statistics. The analysis combined descriptive and inferential statistical methods. Descriptive statistics included frequencies, percentages, mean values (M), and standard deviations (SD) to provide an overview of the sample structure and key variables. To examine relationships between variables, the Pearson correlation coefficient (r) was applied. Differences in consumer attitudes across socio-demographic groups, particularly age and income categories, were analysed using one-way analysis of variance (ANOVA). In addition, hierarchical cluster analysis based on Ward's method was used to identify patterns in the consumption of different product categories. The level of statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Socio-demographic characteristics of the respondents

The socio-demographic characteristics of the respondents were examined to gain insight into the sample structure. The sample was predominantly composed of female respondents (70%), with the largest share belonging to the middle - aged group (34 – 50 years). In terms of income, the majority of respondents fall within the middle - income category, while a smaller proportion belongs to higher and lower income groups. The sample is also characterized by a relatively high level of education. The socio-demographic characteristics of respondents are presented in Table 1 and 2.

Table 1. Attitudes toward factors influencing purchasing in relation to age group

Age group / Attitudes toward purchasing factors	N	M	SD	F	df	Sig.
Young respondents (15–30 years)	48	17,10	4,22	3,126	2	0,047*
Middle-aged respondents (31–50 years)	67	18,06	5,03			
Older respondents (over 50 years)	35	19,80	5,38			
Total	150	18,16	4,94			

Source: Author's calculation based on survey.

Table 2. Attitudes toward purchasing factors in relation to monthly income

Income group / Attitudes toward purchasing factors	N	M	SD	F	df	Sig.
Low level income up to 2,000 BAM	65	16,85	5,35	4,364	2	0,014*
Middle-level income from 2,001 to 3,000 BAM	43	18,91	4,49			
High-level income more than 3,000 KM	42	19,43	4,28			
Total	150	18,16	4,94			

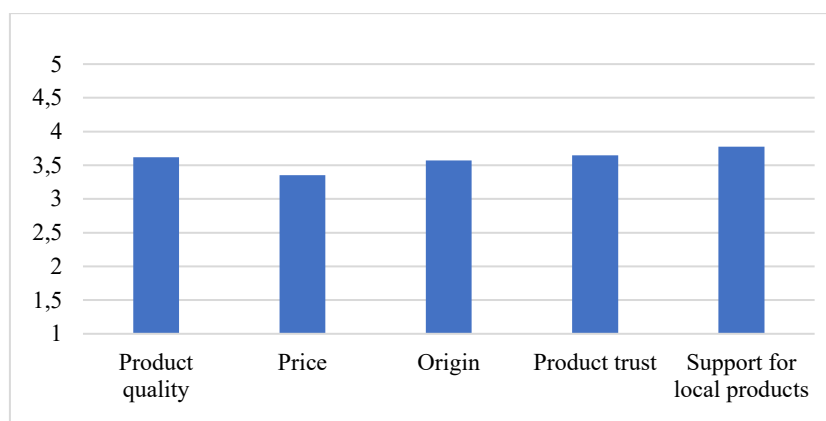
Source: Author's calculation based on survey.

The results of ANOVA test (Table 1.) indicate statistically significant differences in attitudes toward purchasing factors across age groups ($F = 3.126$; $p = 0.047$). The mean values increase with age, suggesting that older respondents express more positive attitudes toward food products compared to younger consumers. This finding may be explained by greater health awareness and stronger attachment to traditional consumption patterns among older consumers. It is also consistent with previous studies indicating higher levels of consumer ethnocentrism and greater importance of product origin in older age groups (Marinković et al., 2011; Jelić Milković et al., 2019).

The results of the ANOVA test (Table 2.) indicate statistically significant differences in attitudes toward purchasing factors across income groups ($F = 4.364$; $p = 0.014$). The mean values increase with income, suggesting that respondents with higher income levels express more positive attitudes toward local food products. This finding indicates that financial capacity plays an important role in shaping consumer behaviour, as higher-income consumers are more likely to prioritize product quality, trust in producers, and support for local production.

Key factors influencing purchasing decisions

The analysis shows that respondents assign the highest importance to supporting local producers ($M = 3.77$), followed by trust in producers ($M = 3.64$) and perceived product quality ($M = 3.62$). Product origin has a moderate level of importance ($M = 3.57$), while price is considered the least important factor (Graph 1).

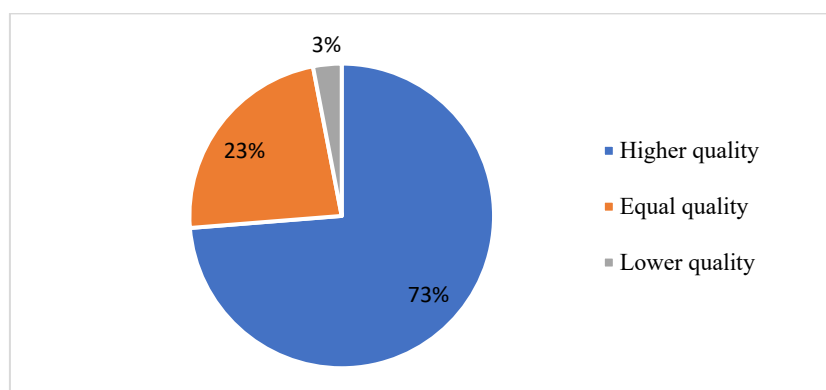


Graph 1. Factors Influencing the purchase of local food products.
Authors based on survey.

These results indicate that consumer decisions are primarily driven by attitudinal factors rather than purely economic considerations. In particular, trust and perceived quality emerge as central determinants of purchasing behaviour, reflecting the importance of direct relationships between producers and consumers within local food systems. This finding is consistent with previous studies emphasizing that quality perception and trust are key elements in the development and sustainability of local food markets (Čivić & Čilimković, 2017; Zaninović & Pavlić Skender, 2025).

Perception of the product quality

The majority of respondents (73%) perceive local food products as being of higher quality than imported alternatives, while a smaller proportion considers them to be of equal quality (Graph 2).



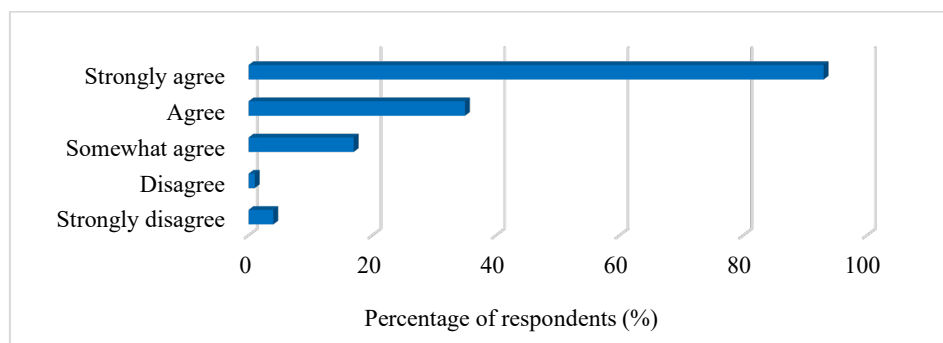
Graph 2. Consumer's perception of the quality of local food products.
Authors based on survey.

This result confirms the strong positive perception of domestic food products and highlights quality as a key driver of consumer behaviour. The perceived superiority of local products may be associated with freshness, authenticity, and trust in production processes. These findings are in line with previous research (Chambers et al., 2007; Ivanković & Radovanović, 2014), which indicates that consumers across different contexts often associate local food with higher quality, even when actual purchasing behaviour may not fully reflect these perceptions.

Purchasing power and consumer behaviour

The results presented in Graph 3 indicate that 62% of respondents fully agree that purchasing power represents a key factor influencing food purchasing decisions and the development of local production. This finding highlights the importance of economic factors in shaping consumer behaviour, suggesting that higher purchasing power enables more frequent purchase of higher-quality, and often more expensive local food

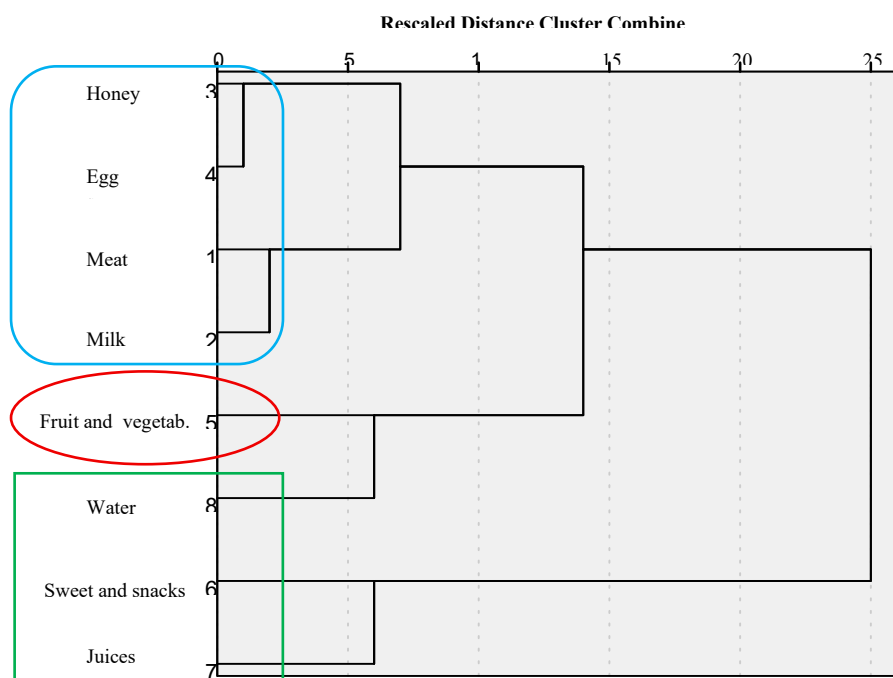
products. At the same time, it indicates that, despite positive attitudes toward local products, actual purchasing decisions remain constrained by economic factors and product availability. The Pearson correlation coefficient between purchasing power and food consumption is high ($r = 0.92$), indicating a strong positive relationship. Overall, the findings confirm that purchasing power represents a key determinant of consumer behaviour, which is consistent with previous research emphasizing the role of socio-economic factors in shaping food consumption patterns (Čivić & Čilimković, 2017; Gligorović, 2024).



Graph 3. Importance of purchasing power in food purchasing decisions.
Authors based on survey.

Consumer segmentation (cluster analysis)

Using Ward's hierarchical cluster analysis, three distinct groups of products were identified based on consumer purchasing patterns (Dendrogram 1).



Dendrogram 1. Hierarchical clustering of local food products based on consumer preferences. Authors based on survey.

The first cluster includes traditional and frequently consumed local products such as honey, eggs, meat, and dairy products. These products are characterized by a high level of consumer trust and regular consumption, indicating strong consumer loyalty. The second cluster consists of fruit and vegetables, which show moderate levels of association with local purchasing patterns. Their consumption appears to be influenced by seasonal availability and market supply, resulting in less consistent purchasing

behaviour. The third cluster includes processed products such as water, sweets, snacks, and juices. These products are weakly associated with local origin and form a separate group, reflecting different consumption patterns and lower levels of attachment to local production. Overall, the results suggest that consumer preferences for local food products vary significantly across product categories, with traditional and minimally processed products showing the highest level of preference and trust.

CONCLUSION

The findings of this study indicate that local food products in the Banja Luka area are characterized by a high level of consumer trust and a strong perception of higher quality compared to imported products. Consumer preferences are not driven solely by quality considerations, but also by the willingness to support local producers, which emerges as one of the key motivating factors. The results further reveal significant differences across demographic groups, with older and higher-income respondents showing stronger preferences for local food products. The strong positive relationship between purchasing power and food consumption confirms that economic capacity remains a crucial determinant of actual purchasing behaviour, despite the presence of favourable attitudes toward local products. The cluster analysis highlights the existence of distinct consumption patterns across product categories, with traditional and minimally processed products demonstrating the highest levels of consumer trust and loyalty, while processed products show weaker associations with local origin.

Overall, the results suggest that the development of local food markets in Bosnia and Herzegovina depends not only on maintaining product quality and consumer trust, but also on improving the economic accessibility of these products. Strengthening direct links between producers and consumers through short food supply chains, along with aligning market supply with the purchasing capacity of households, may contribute to transforming positive consumer perceptions into consistent purchasing behaviour.

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REFERENCES

- Akbarov, S. (2022). Consumer ethnocentrism and purchasing behavior: Moderating effect of demographics. *Journal of Islamic Marketing*, 13 (4), 898-932. <https://doi.org/10.1108/JIMA-02-2020-0047>
- Altıntaş, M.H., & Tokol, T. (2007). Cultural openness and consumer ethnocentrism: An empirical analysis of Turkish consumers. *Marketing Intelligence & Planning*, 25(4), 308-325. <https://doi.org/10.1108/02634500710754565>
- Ben Hassen, T., El Bilali, H., Allahyari, M.S., Karabašević, D., Radosavac, A., Berjan, S., Vaško, Ž., Radanov, P., & Obhodaš, I. (2021). Food Behavior Changes during the COVID-19 Pandemic: Statistical Analysis of Consumer Survey Data from Bosnia and Herzegovina. *Sustainability*, 13(15), 8617. <https://doi.org/10.3390/su13158617>
- Candan, B., Aydın, K., & Yamamoto, G. T. (2008). A research on measuring consumer ethnocentrism of young Turkish customers purchasing behaviors. *Serbian Journal of Management*, 3(1), 39-60.
- Chambers, S., Lobb, A., Butler, L., Harvey, K., & Traill, W. B. (2007). Local, national and imported foods: a qualitative study. *Appetite*, 49 (1), 208-213. <https://doi.org/10.1016/j.appet.2007.02.003>
- Čivić, B., & Čilimković, D. (2017). Characteristics of Consumers' Behavior in Shopping of Food Products in the Market of Bosnia and Herzegovina. *Research in World Economy*, 8(2), 49-58. <https://doi.org/10.5430/rwe.v8n2p49>
- Čutura, M. (2012). The influence of ethnicity on consumers' purchase patterns in the context of Bosnia and Herzegovina. *Economic research-Ekonomska istraživanja*, 2, 83-102.

- Čutura, M., & Arslanagić-Kalajdžić, M. (2023). Potrošački etnocentrizam u teoriji i praksi poticanja kupovine domaćih proizvoda. Mostar: Federalno ministarstvo obrazovanja i nauke.
- Gligorović, K. (2024). Valicon: Promene u ponašanju potrošača u regionu. (online), Available at: <https://www.instore.rs/sr/article/91451/valicon-promene-u-ponasanju-potrosaca-u-regionu>
- Ivanković, R., & Radovanović, N.M. (2014). Ponašanje potrošača u Maxi centru Trebinje. *Tehnika*, 14(5), 883-890. <https://doi.org/10.5937/tehnika1405883I>
- Jalić, N., Antelj, J., Baćević, A., Ivković, M., Čerketa, N., Vaško, Ž., Ostojić, A., Drljača, B., Nikolić, P. (2024). Consumer preferences about fruit brandies. XIII International Symposium on Agricultural Sciences AgroReS 2024, Book of Proceedings, 257-266. <https://doi.org/10.7251/ZARS2401257J>
- Janssen, M., & Jager, W. (2002). Stimulating Diffusion of Green Products. *Journal of Evolutionary Economics*, 12 (3), 283-306. <http://dx.doi.org/10.1007/s00191-002-0120-1>
- Jelić Milković, S., Lončarić, R., & Sabljak, A. (2019). Izravna prodaja ekoloških proizvoda. *Agriculture in Nature and Environment Protection*. Osijek, 242-247.
- Kasumović, M., & Meholjić Kalajdžić, S. (2013). Struktura tržišta osobne potrošnje u Bosni i Hercegovini. *Ekonomski vjesnik*, 26(2), 410-426.
- Kavak, B., & Gumusluoglu, L. (2007). Segmenting food markets - the role of ethnocentrism and lifestyle in understanding purchasing intentions. *International Journal of Market Research*, 49(1), 71-94. <https://doi.org/10.1177/147078530704900108>
- Kiriri, P.N. (2019). Consumer ethnocentrism and attitudes towards local products: A case of Kenyan consumers. *University Journal*, 1(3), 1-18. <https://doi.org/10.59952/tuj.v1i3.20>
- Marinković, V., Stanišić, N., & Kostić, M. (2011). Potrošački etnocentrizam građana Srbije. *Sociologija*, 53(1), 43-58. <https://doi.org/10.2298/SOC1101043M>
- Matić, M. (2013). The impact of demographic and socio-psychological factors on consumers ethnocentric tendencies in Croatia. *Economic Research-Ekonomska Istraživanja*, 26(3), 1-14. <https://doi.org/10.1080/1331677X.2013.11517618>
- O'Cass, A., & Kenny, L. (2002). Understanding the younger Singaporean consumers' views of Western and Eastern brands. *Asia Pacific Journal of Marketing and Logistics*, 14(4), 54-79. <https://doi.org/10.1108/13555850210764954>
- Othman, M.N., Ong, F.S., & Wong, H-W. (2008). Demographic and Lifestyle Profiles of Ethnocentric and Non-Ethnocentric Urban Malaysian Consumers. *Asian Journal of Business and Accounting*, 1(1), 5-26.
- Ramsaran-Fowdar, R. (2010). Are Males and Elderly People more Consumer Ethnocentric? *World Journal of Management*, 2(1), 117-129.
- Rizvić, V., & Agić, Dž. (2022). Priručnik o pravilnim ekološkim izborima u svakodnevnom životu. Tuzla: Fondacija tuzlanske zajednice.
- Shimp, T.A., & Sharma, S. (1987). Consumer Ethnocentrism: Construction and Validation of the CETSCALE. *Journal of Marketing Research*, 24(3), 280-289. <https://doi.org/10.2307/3151638>
- Šapić, S., & Golo, J. (2017). Uticaj dimenzija kulture na stavove i namere potrošača prema stranim i domaćim proizvodima. *Marketing*, 48(4), 235-242. <https://doi.org/10.5937/Markt1704235S>
- Veljković, S. (2009). Uticaj etnocentrizma na potrošače u Srbiji. *Marketing*, 40(2), 97-106.
- Wanninayake, W.M.C.B., & Chovancová, M. (2012). Store personality and behavioral intentions of customers: a comparative study of retail industry in the Czech Republic and Sri Lanka. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 60(7), 477-484. <https://doi.org/10.11118/actaun201260070477>
- Zaninović, P. A., & Pavlić Skender, H. (2025). Kratki opskrbni lanci hranom – preferencije potrošača. Poglavlje u knjizi: Poljoprivreda u perspektivi održivosti. Rijeka, 263-277.
- Zarić, V., Vlahović, B., & Petrović, M. (2016). Local food systems and consumer preferences in Serbia. *Economics of Agriculture*, 63(3), 829-844.

The impact of storage capacity on the integration of local foods in school catering: Evidence from the Czech Republic

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Abstract

This paper aims to assess how storage capacity affects the integration of regional foods into school catering, drawing on empirical evidence from the South Moravian Region of the Czech Republic. The analysis is based on 216 valid responses (41.5% of the targeted population) gathered via a questionnaire distributed electronically to representatives of school canteens in the region. The analysis examines two key variables: perception of sufficiency of storage facilities in school kitchens and the willingness of school canteens to increase the share of local food in students' meals in the future, evaluated using the chi-square test of independence. The calculated test criterion reaches 7.386, which exceeds the critical values for both the 5% significance level (3.84) and the stricter 1% significance level (6.635). The strength of this association was quantified by Cramér's V coefficient, which is recorded at 0.185. This indicates a detectable but weak link between adequate storage capacity and the willingness of school canteens to integrate regional foods into their operations. These results suggest that while physical infrastructure and storage facilities are important factors for shortening food supply chains in the South Moravian Region, they are not the sole determinants; however, modernising and expanding storage capacities appear to be a vital step toward the successful implementation of local foods in school catering in the South Moravian Region.

Keywords: short food supply chains, farms, farm-to-school, barriers, infrastructure, storage facility

INTRODUCTION

Short food supply chains (SFSC) and integration of local foods in school catering are two aspects of the alternative agrifood system that have received a great deal of attention recently from scholars and practitioners (Conner et al., 2011; Bobronnikov, 2021) because of their potential to contribute to the regional economies and food system resilience (Smedzik-Ambrozy et al., 2025; Svobodova et al., 2025; Westerdahl et al., 2025). Also, it is in line with the EU (European Commission, 2020) initiatives such as the Farm to Fork Strategy, that is indicating pathways enhancing sustainable development of European food systems.

The original conceptualisation of SFSCs was done by Marsden et al. (2000) and Renting et al. (2003). Over time, SFSC has gained significant prominence, particularly in the realm of policymaking. A report by the United Nations Industrial Development Organization (UNIDO, 2020) outlines six broad operational types of SFSCs: 1) on-farm sales, 2) farmers' markets, 3) farmers' collective retail spaces and box schemes, 4) consumer-driven initiatives, 5) sales to the HoReCa sector, and 6) public procurement models. Public

procurement models involve the sale of agricultural products to the institutional catering sector, such as schools and hospitals. These farm-to-school (FTS) models can create robust economic channels for local producers while strengthening community food security by providing populations with quality and nutritious meals. FTS initiatives typically integrate local food procurement with interactive learning to improve student nutrition. These educational components often detail the origins of food and the people who produce it, thereby cultivating the bond between students and the agricultural community (Allen & Guthman, 2006). The potential of this model to sustain demand for alternative agri-food products is significant, primarily due to the scale of the Czech school catering system. It serves nearly 2 million meals daily (approximately 20% of the population), with state operating expenditures alone exceeding EUR 260 million in 2024 (CSU, 2025). Combined with new legislation (MSMT, 2025a; MSMT, 2025b; MSMT, 2025c), effective from 2025, which prioritises fresh and minimally processed ingredients in Czech school catering, this segment represents a stable and high-volume market for local producers.

Nonetheless, recent research (Zdrahal et al., 2024) also suggests a set of barriers found in SFSC implementation. Specifically in FTS efforts, Conner et al. (2011) point to 1) lack of a reliable supply of consistently quality products, 2) logistical difficulties and high transaction costs, 3) reliance on processed rather than whole and/or raw products, and 4) difficulties in creating seasonal menus using regional products. Love et al. (2020) summarised sources documenting FTS procurement barriers as 1) availability, 2) price and budget constraints, 3) communication barriers between FTS managers and producers, 4) lack of regional supply chain infrastructure, and 5) concerns regarding local producers' food safety practices.

Previous studies (e.g. Aliyar et al., 2015; Roche et al, 2015) have noted the vast difference in school food environments across different countries and found that there is no uniformity in the provision of school-provided meals across countries. Although studies (Izumi et al., 2010; Gaddis & Coplen, 2018; Hawkins, 2023; Miller et al., 2025) identify inadequate storage and refrigeration as a logistical hurdle for local food procurement in schools, researchers have largely overlooked transitional economies with unique historical developments in their food and education systems. To address this research gap, this paper evaluates how storage capacity affects the integration of local foods into school catering, drawing on empirical evidence from the South Moravian Region of the Czech Republic.

MATERIAL AND METHODS

Hypothesis Development

The integration of local foods into school catering is widely recognised as a key strategy for fostering sustainable public food procurement. However, translating this policy goal into practice is often hindered by physical and infrastructural barriers within schools, particularly in logistics, distribution, and storage (Gaitán-Cremashi et al., 2024).

Existing literature (Gaddis and Coplen, 2017) provides evidence of a shift in many school canteens toward a heat-and-serve practice. Driven by financial pressures and the pursuit of economic efficiency, many institutional kitchens have become highly dependent on pre-packaged, highly processed foods sourced through conventional, globalised supply chains. In school catering, local foods often require different handling than processed, frozen, or shelf-stable goods from large-scale distributors (Lahtinen, 2012; Barbosa Jr. et al., 2022). Incorporating farm-fresh raw materials into school menus requires significant physical space and, crucially, sufficient refrigerated storage capacity (Izumi et al. 2010). Also, storage isn't just about space; it's about the type of space (Miller et al., 2025). Local foods often arrive dirty or unprocessed (Thompson et al. 2016), requiring separate storage to prevent cross-contamination, which older facilities may not support. The path dependency principle (David, 1985) suggests that the historical infrastructure determines current constraints. When schools lack the physical and adequate space to safely store bulk purchases of fresh local food, they are forced to adapt their procurement strategies, often relying on more just-in-time deliveries (Lahtinen, 2012). This lack of adequate on-site storage or refrigeration space shifts the logistical burden onto the local farmers. For small- and mid-scale regional producers, managing frequent, small-volume deliveries to multiple school sites generates prohibitively high transaction costs and represents a primary obstacle to integration of local foods in school catering (Izumi et al., 2010).

Due to these spatial and technological limitations, school catering operators often adopt risk-averse purchasing behaviours. To bypass the logistical complexities, coordination efforts, and spoilage risks associated with local food, schools frequently default to conventional wholesale items that do not require any alterations to their existing storage, transport, or cooking routines (Izumi et al., 2010; Barbosa Jr. et al., 2022). Also, the school catering relies on large-volume storage to lower costs, but integrating local foods shifts the focus to the problem of achieving the economies of scope (variety and freshness). Institutional theory (Scott, 2014) suggests that organisations (like schools) are governed by sets of rules and norms that prioritise efficiency and risk mitigation. This implies that even when procurement laws are changed to favour local sourcing, the physical infrastructure (storage) could remain the missing link that prevents policy from becoming practice. Therefore, the availability of adequate storage capacity is a structural determinant for shortening food supply chains and integrating local food into the school catering. Based on this summary of the existing knowledge, we propose to formally test the following hypothesis.

Null Hypothesis (H_0): There is no positive relationship between the available storage capacity of school canteens and their intention to increase the share of local food in the meals they prepare.

Data and Methods

The analysis is based on primary data from quantitative questionnaire survey conducted among school canteens of primary schools in the South Moravian Region of the Czech Republic. The questionnaire was distributed electronically to representatives of all school canteens in the target population. The survey targeted the entire population of school canteens in the region, identified through the official register of educational institutions. In total, 521 school canteens were contacted; first in the Brno Metropolitan area in 2025 and in the rest of the South Moravian Region in 2026. For the present analysis, 216 valid responses were available, corresponding to 41.5% of the contacted population. For a population of this size, commonly used sample size guidelines suggest approximately 220 responses for a 95% confidence level and a 5% margin of error. The analysis is based on 216 valid responses, which is close to this threshold and provides a reasonable basis for identifying general patterns in the analysed relationship. Accordingly, the present analysis examines two key variables: perception of sufficiency of storage space in school kitchens and the willingness of school canteens to increase the share of local food in the future. The relationship between these variables was evaluated using the chi-square test of independence. The strength of the association was assessed using Cramér's V coefficient.

RESULTS AND DISCUSSION

Based on the empirical evidence from school canteens in the South Moravian Region, the following figures (Figure 1a and 1b) illustrate the perception of storage capacity of these facilities and their strategic intentions regarding local food procurement. The school canteens mostly report a sufficient and adequate storage space (67.6%). Among school canteens, the 69.4% of them expressed a plan to increase the share of local foods in their prepared dishes. This highlights that the willingness to participate in farm-to-school initiatives is high.

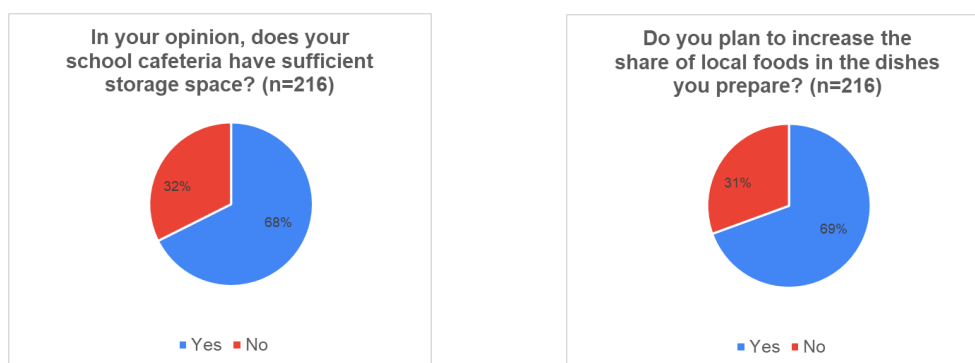


Figure 1 a and 1 b.

Source: The authors' construct based on data from the survey

The following figure (Figure 2) demonstrates what perceived storage sufficiency has on the actual operational capabilities of school canteens to handle fresh produce (using the example of fresh fruits and vegetables). For canteens reporting sufficient storage space, the majority are capable of storing fresh fruits and vegetables for a week, whereas those with insufficient space are restricted to much shorter windows, often limited to just one day or requiring frequent, immediate deliveries. This indicates that the physical infrastructure may appear as a structural barrier that may prevent policy goals from being translated into practice in the South Moravia.

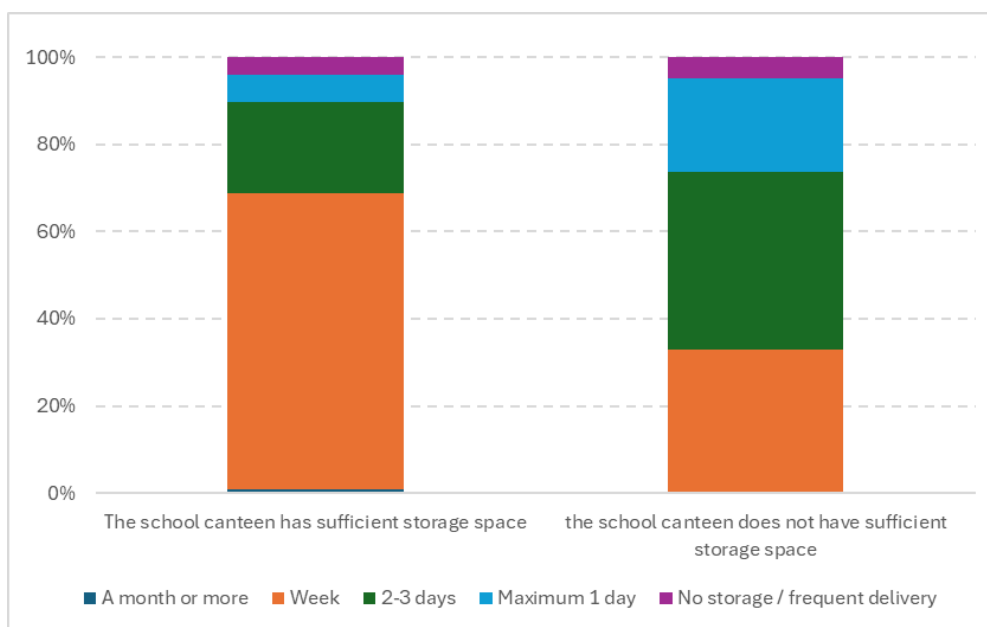


Figure 2: The ability of analysed schools to store fresh fruits and vegetables in refrigerated areas
Source: The authors' construct based on data from the survey

In a further step, we examined the relationship between the perceived sufficiency of storage space in school canteens and their intention to increase the proportion of local foods in the meals they prepare. Based on the contingency table of respondents from the South Moravian Region, 110 out of 146 canteens (75.3%) have a sufficient storage plan to increase their use of local products. Of the 70 canteens with insufficient storage, only 40 plan (57.1%) to increase regional food use.

The statistical evaluation using the chi-square test of independence confirms the existence of a relation between the tested variables. The calculated test criterion reaches 7.386, which exceeds the critical values for both the 5% significance level (3.84) and the stricter 1% significance level (6.635). The strength of this association was quantified by Cramér's V coefficient, which is recorded at 0.185. In the context of statistical measurements, this indicates a detectable but weak link between adequate storage capacity and the willingness of school canteens to integrate regional foods into their operations.

CONCLUSION

This paper evaluates how storage capacity affects the integration of local foods into school catering, drawing on empirical evidence from the South Moravian Region of the Czech Republic. These results suggest that while storage facilities are factors for shortening food supply chains in the South Moravian Region, they are not the sole determinants; however, modernising and expanding storage capacities appear to be a vital step toward the successful implementation of local foods in school catering.

This result supports the path dependency principle cited in the research, suggesting that historical infrastructure constraints dictate current procurement limits. Specifically, the lack of refrigerated areas forces schools into a more just-in-time delivery model, which shifts the logistical burden onto local farmers and creates a primary obstacle to integrating local foods due to high transaction costs.

The study contributes to the global knowledge base by providing additional geographical evidence that suggests physical infrastructure is serving as a structural determinant for the success of FTS initiatives in transitional economies like the Czech Republic. The study also contributes to the local knowledge base by providing the first analysis of barriers in school catering and quantifying the specific operational constraints faced by these facilities. The Czech school catering system can serve as a stable and high-volume market for local producers if structural limitations are addressed.

Despite its merits, the study has several limitations. First, the analysis is based on self-reported data, which may be subject to response bias. Second, although the concept of local food is discussed in the literature (Martinez et al., 2010; Granvik et al, 1981), it is not uniformly defined and may be interpreted differently by respondents, which could affect the consistency of the responses.

Further research should identify other factors that serve as barriers to integrating local food into school canteens in the South Moravian Region, as well as address the complexity and interplay of these factors.

ACKNOWLEDGEMENT

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REFERENCES

- Aliyar, R., Gelli, A. & Hamdani, S. H. (2015). A review of nutritional guidelines and menu compositions for school feeding programs in 12 countries. *Frontiers in Public Health*, 3, 148. <https://doi.org/10.3389/fpubh.2015.00148>
- Allen, P., & Guthman, J. (2006). From “old school” to “farm-to-school”: Neoliberalization from the ground up. *Agriculture and Human Values*, 23(4), 401–415. <https://doi.org/10.1007/s10460-006-9019-z>
- Barbosa Jr., R., Coca, E. & Soyer, G. (2022). School food at home: Brazil’s national school food programme (PNAE) during the COVID-19 pandemic. *Social & Cultural Geography*, 24(3–4), 620–639. <https://doi.org/10.1080/14649365.2022.2115538>
- Bobronnikov, E., Boyle, M., Grosz, M., Lipton, I., Nutter, R., Velez, M., & Yadav, L. (2021). Farm to School Literature Review. Rockville: Abt Associates. <https://1url.cz/FeEAA>
- Conner, D. S., Nowak, A., Berkenkamp, J., Feenstra, G. W., Van Soelen Kim, J., Liquori, T., & Hamm, M. W. (2011). Value chains for sustainable procurement in large school districts: Fostering partnerships. *Journal of Agriculture, Food Systems, and Community Development*, 1(4), 55–68. <https://doi.org/10.5304/jafscd.2011.014.005>
- CSU. ČESKÝ STATISTICKÝ ÚŘAD (2025). Výdaje na vzdělávání v ČR: Veřejné výdaje na školství (kapitola Stravování žáků). Retrieved from: <https://csu.gov.cz/vydaje-na-vzdelavani>
- David, P.A. (1985). Clio and the Economics of QWERTY. *American Economic Review*, 75(2), 332–337.
- European Commission (2020). *A Farm to Fork Strategy for a Fair, Healthy and Environmentally-Friendly Food System*. Brussels: European Commission.
- Gaddis, J. & Coplen, A. K. (2018). Reorganizing School Lunch for a More Just and Sustainable Food System in the US. *Feminist Economics*, 24(3), 89–112. <https://doi.org/10.1080/13545701.2017.1383621>
- Gaitán-Cremaschi, D., Valbuena, D. & Klerkx, L. (2024). The roles and dynamics of transition intermediaries in enabling sustainable public food procurement: insights from Spain. *Agriculture and Human Values*, 41, 1591–1615. <https://doi.org/10.1007/s10460-024-10562-7>
- Granvik, M., Joose, S., Hunt, A. & Hallberg, I. (2017). Confusion and misunderstanding—Interpretations and definitions of local food. *Sustainability*, 9(11), 1981. <https://doi.org/10.3390/su9111981>
- Hawkins, R. (2023). Spatial barriers to the effective delivery of school food policy in UK primary schools: Findings from an Institutional Ethnography. *People, Place and Policy*, 17(2), 59–81. <https://doi.org/10.3351/ppp.2023.5335632665>

- Izumi, B. T., Wright, D. W., & Hamm, M. W. (2010). Market diversification and social benefits: Motivations of farmers participating in farm to school programs. *Journal of Rural Studies*, 26(4), 374–382. <https://doi.org/10.1016/j.jrurstud.2010.02.002>
- Lahtinen, U. (2012). Sustainability and local food procurement: a case study of Finnish public catering. *British Food Journal*, 114(8), 1053–1071. <https://doi.org/10.1108/00070701211252048>
- Love, E., McFadden, D. T., Jablonski, B. R. R., Bellows, L. (2020). A baseline assessment of school food spending and local procurement: Exploring the case of CO HB 19-1132 and other public policies. Selected Paper prepared for presentation at the 2020 Agricultural & Applied Economics Association Annual Meeting, Kansas City. Retrieved from: <https://1url.cz/qeBgS>
- Marsden, T., Banks, J., & Bristow, G. (2000). Food supply chain responses to the food crisis: institutional or market-based responses?. *Sociologia Ruralis*, 40(4), 424–438. <https://doi.org/10.1111/1467-9523.00158>
- Martinez, S., Hand, M. S., Pra, M. D., Pollack, S., Ralston, K., Smith, T., Vogel, S., Suttles, S., Lohr, L., Low, S. & Newman, C. (2010). *Local Food Systems: Concepts, Impacts, and Issues*. Washington, DC: USDA Economic Research Service.
- Miller, E. T., Schroeder, K., Thapa, B., & Shay, E. (2025). Barriers to access to local produce in an early childhood setting in rural Appalachia. *Journal of Agriculture, Food Systems, and Community Development*, 14(1), 169–183. <https://doi.org/10.5304/jafscd.2024.141.004>
- MSMT. MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY (2025a). MŠMT představuje novinky ve školním stravování: zdravější jídla, větší podpora jídelen. Retrieved from: <https://1url.cz/LeEA6>
- MSMT. MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY (2025b). Změna financování nepedagogické práce v souvislostech. Retrieved from: <https://1url.cz/SeEAX>
- MSMT. MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY (2025c). Školní stravování: Výkonová data za školní rok 2024/2025. Retrieved from: <https://1url.cz/yeEA8>
- Scott, W. R. (2014). *Institutions and organizations: Ideas, interests, and identities*. 4th ed. Thousand Oaks, CA: SAGE Publications.
- Smedzik-Ambrozy, K., Sapa, A., Borychowski M., Stepien, S., Svobodova, E., Zdrahal, I., Lategan, F. & Grega, L. (2025). Do short food supply chains impact on efficiency of farms? Evidence from Poland and Czechia. *Studies in Agricultural Economics*, 127(3), 152–161. <https://doi.org/10.7896/j.3109>
- Svobodova, E., Zdrahal, I., Dankova, B., Tkacova, M., Raszkova, S. & Somerlikova, K. (2025). The Size Factor in the Inclusion of Regional Foods in School Canteen: An Empirical Analysis. *Book of Proceedings of the 19th International Scientific Conference, INPROFORUM 2025*, 370–375. <https://doi.org/10.32725/978-80-7694-143-4.46>
- Renting, H., Marsden, T. K., & Banks, J. (2003). Understanding alternative food networks: exploring the role of short food supply chains in rural development. *Environment & planning A*, 35(3), 393–411. <https://doi.org/10.1068/a3510>
- Roche, E., Conner, D., & Kolodinsky, J. (2015). Increasing Local Procurement in Farm to School Programs: An Exploratory Investigation. *Journal of Agriculture, Food Systems, and Community Development*, 5(2), 81–90. <https://doi.org/10.5304/jafscd.2015.052.019>
- Thompson, J. J., Brawner, A. J. & Kaila, U. (2017). “You can’t manage with your heart”: risk and responsibility in farm to school food safety. *Agriculture and Human Values*, 34, 683–699. <https://doi.org/10.1007/s10460-016-9766-4>
- UNIDO (2020). *Short Food Supply Chains for Promoting Local Food on Local Markets*. Vienna: Austria, retrieved from: <https://hub.unido.org/node/2879>
- Westerdahl, S., Pugh, R., Nordstrom Kallstron, H., & Krautscheid, L. (2025). Local food and sustainable regional development. *Regional Studies*, 59(1), 2581127. <https://doi.org/10.1080/00343404.2025.2581127>
- Zdrahal, I., Stepien, S., Lategan, F. S., Svobodova, E., Borychowsky, M., Sapa, A., Smedzik-Ambrozy, K. & Grega, L. (2024). Barriers to participate in short food supply chains: preliminary results from the survey among farmers in Poland and the Czech Republic. *Book of Proceedings of XIII International Symposium on Agricultural Sciences, AgroRes 2024*. 230–237. Retrieved from: <https://1url.cz/AeEmi>

Green management as a tool supporting the sustainable development of the food value chain in agriculture

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Abstract

The sustainable development of the agri-food sector, particularly within the food value chain, faces increasing pressure due to significant levels of food loss and waste, as well as rising environmental and climate-related challenges. Current estimates indicate that food waste constitutes a substantial economic burden and contributes notably to greenhouse gas emissions, highlighting the urgency of implementing more efficient and sustainable management practices across all stages of the supply chain. This paper examines the role of green management as a tool supporting the transition toward a more sustainable and resource-efficient agri-food system. It identifies the main drivers of food loss and waste, grouping them into technical, technological, environmental, economic, and social categories. Importantly, these factors are closely linked to human behavior, decision-making processes, and the level of individual competencies. In this context, the concept of green soft skills is introduced as a critical component enabling sustainable transformation. These skills encompass environmental awareness, responsible attitudes, analytical thinking, and the ability to apply sustainable practices in everyday professional activities. They also include competencies related to resource efficiency, climate change mitigation, and the use of environmentally friendly technologies. The methodology was based on preliminary research on green soft skills using a survey among STEM students from six countries participating in the SOFTEN project. The study highlights the growing demand for green skills in the labor market, particularly in sectors with high environmental impact, such as agriculture and food processing. It also emphasizes the key role of education in equipping current and future professionals with the necessary competencies to support the green transition. The findings suggest that strengthening green soft skills can significantly contribute to reducing food waste, improving resource efficiency, and minimizing the environmental footprint of the food value chain. Ultimately, integrating these competencies into management practices is essential for achieving long-term sustainability development goals (SDG8, SDG9, SDG15) and supporting the transition toward a low-carbon and circular economy (SDG7, SDG12, SDG13).

Keywords: sustainable development, green competences, food value chain, food loss and waste

INTRODUCTION

Food loss and food waste occur at all stages of the food chain: from primary production, through storage, processing, transport and trade, to consumption (Figure 1). According to FAO (2023a), about 13-14% of food is lost before the sale stage, while another about 19% is wasted at the level of trade and consumption, which together means that even close to 1/3 of global food production is not consumed (UNEP, 2024). The scale of this phenomenon is global and systemic, covering all regions of the world, although its causes and structure differ between developed and developing countries. As a result, about 1.3 billion tonnes of food are lost or wasted each year (FAO, 2015).

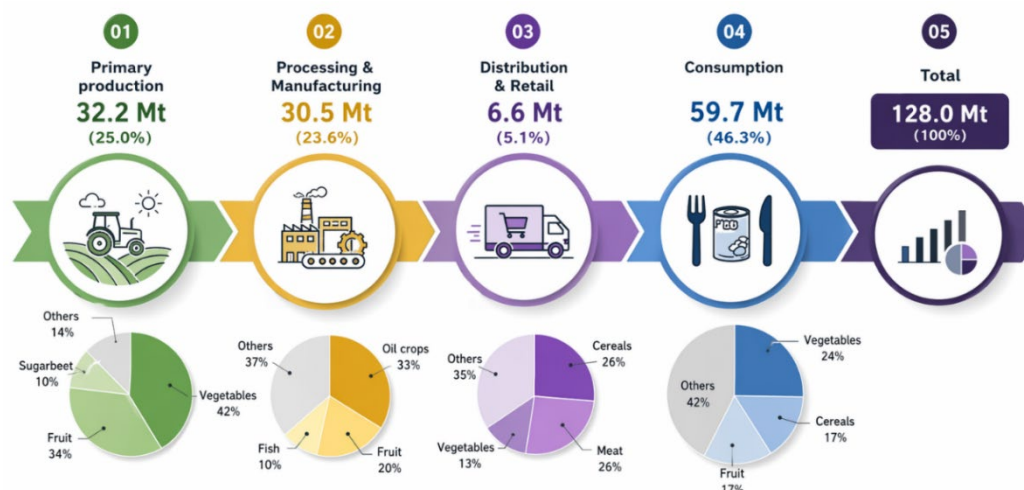


Figure 1. Food loss and waste in the whole value chain (Adapted from Caldeira et al., 2019)

From an economic perspective, food loss and waste also mean very high costs, which are estimated at USD 1-2 per 1 kg, if environmental and social losses (wasted labor, energy, water, fertilizers or capital) are also taken into account (FAO, 2013). In developed countries, most of the food loss and waste are incurred at the consumer and retail level. In developing countries, on the other hand, smallholder farmers are particularly affected, losing income due to dispersion, lack of warehousing, transport infrastructure or market access. This causes a certain paradox, because on the one hand, food is lost/wasted in the supply chain, and on the other hand, there are shortages and hunger in many locations. Figure 2 provides an example map of the degree of food waste in Sub-Saharan African countries.



Figure 2. Food waste in Sub-Saharan Africa (own elaboration based on FAO, 2023b)

It should be noted that the environmental dimension of food loss and waste are equally important. Unused food is responsible for about 8-11% of global greenhouse gas emissions and contributes to the

degradation of ecosystems and biodiversity loss (Darnas et al., 2025; Yin et al., 2026). This means that reducing food loss and waste are one of the key actions in the context of climate policy and the implementation of the Sustainable Development Goals (SDGs). Unfortunately, there are many significant barriers to effective reduction of food loss and waste, which can be divided into several categories (HLPE, 2014):

- technical – lack of appropriate technical solutions (e.g. warehouses, cold storage),
- technological – low level of processing and logistics technology,
- environmental – the impact of climate, seasonality and extreme weather events,
- economic – lack of financial resources, inefficient markets, high investment costs,
- social – consumer habits, lack of awareness and education.

Although these barriers are often analyzed as distinct categories, they are actually strongly linked to the human factor and aspects such as the level of knowledge, skills and competences of those involved in the food chain. This means that even the availability of technology does not guarantee the reduction of losses if there is a lack of competence to use it effectively. In this context, the importance of green soft skills (Cabral and Lochan Dhar, 2019), which include, inter alia, environmental awareness, responsibility, the ability to make decisions that take into account the life cycle of the product, as well as the ability to cooperate and manage in the spirit of sustainable development, is growing. These competencies are particularly relevant in the context of the transformation of the economy towards a low-carbon and resource-efficient model, where the combination of technical knowledge with social and managerial competencies is required (Nikolajenko-Skarbale et al., 2021). These competencies complement technical knowledge and allow for the effective implementation of solutions to reduce food losses. Integrating green competences into management practice (the so-called sustainable green management) makes it possible to take action at every stage of the food chain – from optimizing production, through improving logistics and warehousing, to changing consumer behavior. As a result, this leads to increased resource efficiency, reduced food losses and reduced emissions of pollutants into the atmosphere, which is an important part of the transition towards sustainable food systems (EU and JRC, 2022; Sarkis, 2012; Aschemann-Witzel et al., 2015).

MATERIALS AND METHODS

Against this background, the aim of the study is to assess the current state of awareness of green soft skills among academic communities in selected European countries, with particular focus on STEM (Science, Technology, Economics, Mathematics) education and its role in supporting the sustainable transformation of the food sector. To this end, a survey-based approach was adopted, covering both students and academic teachers across six European Union countries. This allowed for a comparative analysis of the perceived importance of green competences, existing barriers to their integration into study programs, and expectations regarding future education in this area.

The paper's thematic scope covers issues related to the sustainable development of the food value chain in agribusiness using green management and education that supports the acquisition of green soft skills. The survey was conducted as part of international projects SOFTEN (*Embedding Soft Skills in STEM Academic Curriculum for the Transition to Sustainable Green Economy*) and CHAIN (*Cooperation for Holistic Agriculture Innovation Nests in Sub-Saharan Africa*), co-funded by the European Union (SOFTEN, 2025; CHAIN, 2026). The aim of the preliminary surveys was to examine the level of awareness of academia about the role of green soft skills and their importance for the future work of STEM students in green economy sectors. Two separate online questionnaires were developed: one addressed to students in the STEM (Science, Technology, Economics, Mathematics) area, and the other to academic teachers of these fields. The study was conducted in six countries participating in the project: Poland, Greece, Italy, Lithuania, Finland and Portugal. The questionnaires covered three thematic areas: demographics, the level of knowledge about soft skills and their green dimension, the perception of their importance for the transition towards a sustainable economy. Details on the list of questions are included in the report (SOFTEN WP2 Report, 2025).

RESULTS

The final sample size was 98 students and 65 academic teachers. In both cases, the group of respondents was above the sample size planned in the SOFTEN project. The main selection condition for both groups was pursuing studies or teaching in the STEM field. In student surveys, 51% were women, 46% were men; 61% of respondents studied at the bachelor's level, 28% at the master's level. Engineering majors dominated, but the sample also included natural sciences, computer science, mathematics, economics and management. Almost 94% of respondents from both groups studied or lectured at public universities. On the other hand, among academic teachers, the largest group consisted of assistant professors and lecturers (approx. 21.5% each category), professors (20%), assistants (19%) and doctoral researchers (17%). Teaching experience ranged from 2 to 30 years. The sample is therefore well diversified.

Familiarity with the concepts of soft skills and green soft skills

The survey revealed a clear discrepancy in the level of familiarity with soft skills versus green soft skills in both respondent groups (Figure 3). Among STEM students, approximately 66% declared a high level of familiarity with the concept of soft skills (scores 4–5 on a five-point scale), while the corresponding figure for green soft skills dropped to just 33%. A similar pattern was observed among academic staff, where 83% reported being familiar with soft skills in general, but only 42% demonstrated equivalent awareness of their green dimension. Notably, as many as 21% of students assigned the lowest possible score (1) to their familiarity with green soft skills, compared to just 3% for soft skills in general. These findings indicate that, while the concept of soft skills is relatively well established in both groups, its extension to the sustainability domain remains significantly less recognized.

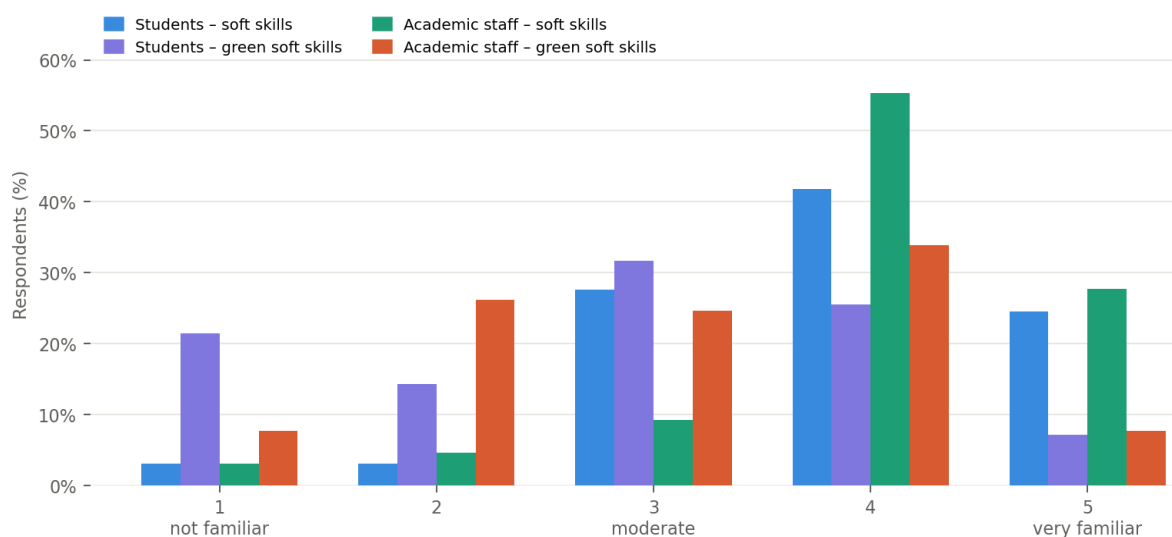


Figure 3. Familiarity with soft skills and green soft skills: students vs academic staff (1–5 scale)

Key competencies for the green economy

When asked to identify the most important soft skills for professional development in green economy sectors, both groups showed notable convergences but also meaningful divergences (Figure 4). Among students, the top-ranked competencies were communication skills (55% of respondents), problem solving (47%), and creativity (45%). Academic staff, by contrast, placed critical thinking first by a large margin (82%), followed by problem solving (62%) and communication skills (59%). A particularly striking gap was observed for environmental empathy, considered important by nearly 48% of academic staff but by only 22% of students. Planning and organizational skills were ranked comparably by both groups (43% students, 42% staff), while team management was valued notably more by academic staff (55%) than by students (36%).

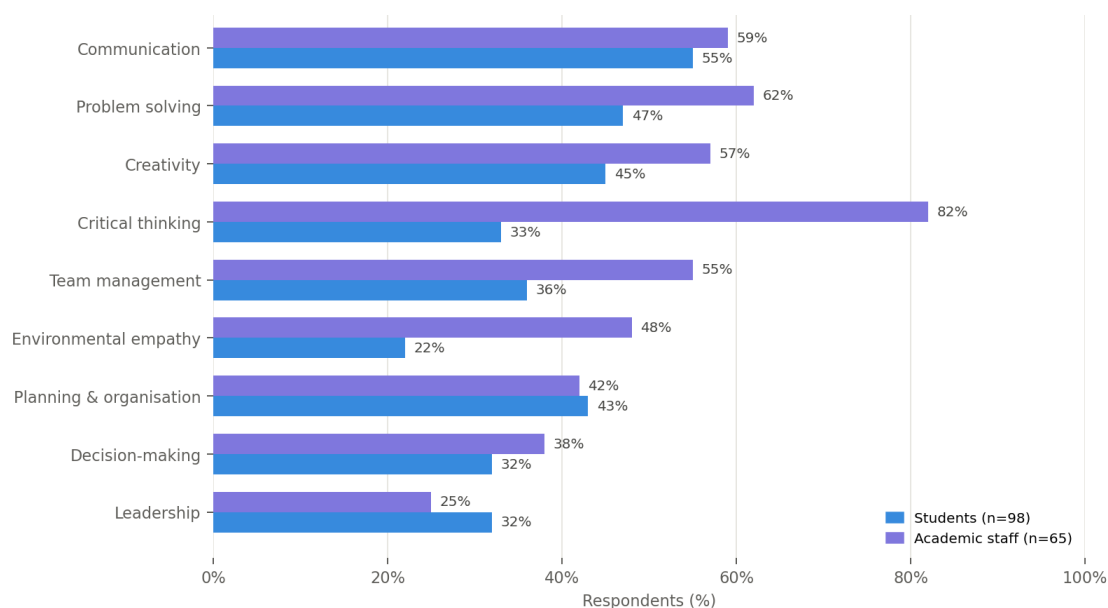


Figure 4. Most important soft skills for the green economy: students vs academic staff (% of respondents, multiple choice)

DISCUSSION

The results reveal a two-tier awareness gap in STEM higher education: a relatively solid general understanding of soft skills coexists with a considerably weaker grasp of their green dimension. This pattern is consistent with findings reported by Nikolajenko-Skarbale et al. (2021), who identified a persistent disconnect between the growing labor market demand for green competencies and their limited integration into university curricula. The low familiarity with green soft skills observed among students (with one in five unable to characterize the concept at all) suggests that the connection between transferable skills and environmental responsibility is not yet effectively communicated within STEM programs.

The divergence in competency hierarchies between students and academic staff is particularly informative. The dominant role assigned to critical thinking by academic staff (82%) aligns closely with the framework proposed by Wiek et al. (2011), who identified system-thinking and normative competence (both closely tied to critical and analytical reasoning) as foundational for sustainability problem solving. Students' focus on communication and creativity, while valuable in professional contexts, reflects a more instrumentally oriented perspective, potentially shaped by perceived employability demands rather than a deep engagement with sustainability challenges. The notably lower student valuation of environmental empathy (22% vs. 48% for staff) is especially significant in the context of the food value chain, where the capacity to recognize and internalize environmental consequences of production, logistics, and consumption decisions is central to effective green management.

From the perspective of the competence framework of Wiek et al. (2011), the skills most valued by academic staff (critical thinking, problem solving, and environmental empathy) map closely onto systems-thinking competence, normative competence, and interpersonal competence, respectively. Students' preferences, weighted towards communication and creativity, relate more directly to strategic and interpersonal competencies in the same framework. This suggests that current STEM education tends to cultivate competencies useful for implementing sustainability strategies, but provides less support for the normative and anticipatory dimensions needed to evaluate and design them. In the context of sustainable food systems, where trade-offs between economic viability, resource efficiency, and ecological impact must be continuously negotiated, this imbalance represents a significant educational gap. Bridging it requires a deliberate curricular shift toward competencies that enable learners not only to act, but also to assess, envision, and justify the direction of that action (Wiek et al., 2011; Cabral and Lochan Dhar, 2019).

CONCLUSION

The findings of this study confirm that green soft skills represent a critical, yet still underutilized, component of sustainable management in the food value chain. Food loss and waste, driven not only by technical and infrastructural deficiencies but fundamentally by human decision-making, competence gaps, and behavioral patterns, cannot be effectively addressed without equipping professionals at every stage of the chain with the capacity to think systemically, act responsibly, and apply sustainability principles in practice.

The survey evidence demonstrates that awareness of green soft skills remains substantially lower than general familiarity with soft skills, both among STEM students and academic staff across six European countries (Poland, Greece, Italy, Lithuania, Finland and Portugal). This gap carries direct consequences for the agri-food sector: graduates entering the food value chain (whether in production, logistics, processing, or retail) bring insufficient competence in environmental empathy, critical thinking, and normative judgement, precisely the skills needed for effective sustainability problem solving.

Higher education institutions, therefore, bear a pivotal responsibility. Integrating green competencies into STEM curricula, not as optional additions but as core elements of the academic programme, is a prerequisite for enabling the subsequent effective implementation of green management practices across the food value chain. Only when future professionals are trained to recognize trade-offs, anticipate environmental consequences, and translate sustainability values into everyday operational decisions will it become possible to meaningfully reduce food losses, improve resource efficiency, and support the transition toward a low-carbon and circular agri-food system, in line with the objectives of SDG8, SDG12, and SDG13.

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REFERENCES

- Aschemann-Witzel, J., De Hooge, I., Amani, P., Bech-Larsen, T., Oostindjer, M. (2015). Consumer-related food waste: Causes and potential for action. *Sustainability*, 7(6), 6457-6477. <https://doi.org/10.3390/su7066457>
- Cabral, C., Lochan Dhar, R. (2019). Green competencies: construct development and measurement validation, *Journal of Cleaner Production*, 235, 887-900. <https://doi.org/10.1016/j.jclepro.2019.07.014>
- Caldeira, C., De Laurentiis, V., Corrado, S., van Holsteijn, F., Sala, S. (2019). Quantification of food waste per product group along the food supply chain in the European Union: a mass flows analysis. *Resources, Conservation & Recycling*, 149, 479-488. <https://doi.org/10.1016/j.resconrec.2019.06.011>
- CHAIN 2026. Erasmus+ Project: Cooperation for Holistic Agriculture Innovation Nests in Sub-Saharan Africa. <https://project-chain.eu/>
- Darnas Y., Sembiring E., Rahardyan B., Erdiwansyah (2025). Food waste reduction efforts in reducing greenhouse gas emissions and the impact of climate change: A review. *Sustainable Chemistry for Climate Action*, 7, 100152. <https://doi.org/10.1016/j.scca.2025.100152>

- European Commission, Joint Research Centre. (2022). GreenComp: The European sustainability competence framework. Publications Office of the European Union. <https://doi.org/10.2760/13286>
- FAO. (2013). *Food Wastage Footprint: Impacts on Natural Resources*. Rome: Food and Agriculture Organization of the United Nations. <https://www.fao.org/4/i3347e/i3347e.pdf>
- FAO. (2015). Food loss and waste facts. <https://openknowledge.fao.org/server/api/core/bitstreams/82e4fdce-6cbb-4837-a615-e249e876acc1/content>
- FAO. (2023a). Indicators. In: Sustainable Development Goals. Rome. <https://doi.org/10.4060/CC7088EN-fig52>
- FAO. (2023b). Food Loss and Waste database. <https://www.fao.org/platform-food-loss-waste/flw-data/en/>
- HLPE. (2014). *Food losses and waste in the context of sustainable food systems*. A report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security, Rome 2014. https://www.fao.org/fileadmin/user_upload/hlpe/hlpe_documents/HLPE_R
- Nikolajenko-Skarbale, J., Viederyte, R., Sneideriene, A. (2021). The Significance of “Green” Skills and Competencies Making the Transition Towards the “Greener” Economy. *Rural Sustainability Research*, 46(341), 53-65. <https://doi.org/10.2478/plua-2021-0017>
- Sarkis, J. (2012). A boundaries and flows perspective of green supply chain management. *Supply Chain Management*, 17(2), 202-216. <https://doi.org/10.1108/13598541211212924>
- SOFTEN 2025. Erasmus+ Project: Embedding Soft Skills in STEM Academic Curricula for the Transition to Sustainable Green Economy. www.softenproject.eu
- SOFTEN WP2 Report 2025. Framework for the integration of green soft skills in STEM education (2025), WP2 Report, https://softenproject.eu/wp-content/uploads/2026/01/SOFTEN_Framework-Report_WP2_2026_EN.pdf
- UNEP. (2024). Food Waste Index Report 2024: Think Eat Save. ISBN: 978-92-807-4139-1. <https://wedocs.unep.org/items/dbe2cd4c-8384-4636-8359-5847f42b9711>
- Wiek, A., Withycombe, L., Redman C.L. (2011). Key competencies in sustainability: a reference framework for academic program development. *Sustainable Science*, 6, 203-218. <https://doi.org/10.1007/s11625-011-0132-6>
- Yin, K., Zhu, J., Wu, M., Zhang, H., Ling, X., Cai, M., Ren, S., Zhao, X., Ling, C., Yu, L., Tong, H., He, C., Wang, Y., & Fei, X. (2026). Misbehaviour dominates GHG emissions from food loss and waste. *Nature Climate Change*, 16, 424–432. <https://doi.org/10.1038/s41558-026-02596-y>

Organic food as a path to sustainable and healthy living: Consumer behavior and market trends

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Abstract

Organic food consumption has emerged as a global phenomenon, reflecting both increasing health consciousness and heightened concern for environmental sustainability. This paper investigates the key drivers of organic food consumption, with particular emphasis on health motivations, food safety concerns, and environmental awareness. The existing body of literature indicates that consumers perceive organic products as safer and more nutritious, primarily due to their production methods that exclude synthetic pesticides, fertilizers, and genetic modification. Health consciousness consistently appears as a central determinant of purchase intention, as consumers seek not only to enhance personal well-being but also to prevent diet-related illnesses such as obesity. Simultaneously, awareness of food safety risks and ecological impacts further strengthens the preference for organic alternatives. By synthesizing market data and consumer behavior research, this paper highlights the relevance of organic food as both an economic opportunity and a public health intervention. The findings suggest that fostering organic consumption can contribute to healthier lifestyles while promoting sustainable agricultural practices.

Keywords: consumer behavior, sustainable agriculture, organic food consumption, health consciousness

INTRODUCTION

In recent decades, the global food industry has witnessed a remarkable surge in the demand for organic products. This trend is driven by growing consumer awareness of the health benefits associated with organic diets and the environmental sustainability of organic farming practices (Latinović et al., 2023). Organic food, produced without synthetic pesticides, fertilizers, or genetic modification, is increasingly perceived as a safer and healthier alternative to conventional products (Rahman et al., 2024). While the organic sector has expanded rapidly in developed nations, its adoption in developing countries remains relatively limited, often constrained by reliance on imports and lower levels of consumer awareness (Ashaolu & Ashaolu, 2020).

The significance of organic food consumption extends beyond market growth. It reflects a broader societal shift toward health consciousness and sustainable living. Organic agriculture has gained significant attention in recent years, not only for its environmental benefits but also for its potential positive impact on human health (Rahman et al., 2024). Rising rates of obesity and diet-related illnesses underscore the urgent need for healthier food options, while consumer concerns about food safety and environmental degradation further reinforce the appeal of organic products. Understanding the motivations behind organic food consumption is therefore essential for both industry stakeholders and policymakers, particularly in developing countries where the market is still emerging (Ostojić et al., 2025).

The aim of this paper is to examine the factors influencing consumer demand for organic food, with emphasis on health motivations, food safety concerns, and environmental awareness. By synthesizing existing literature and market data, the paper highlights the drivers of organic food consumption and explores implications for public health and sustainable food systems. This paper is significant because it bridges consumer behavior, public health, and market development. It underscores the role of organic food as both an economic opportunity and a public health intervention, offering insights into how healthier and more sustainable dietary choices can be promoted.

The paper is structured as follows: the introduction outlines the rapid growth of the global organic food industry and identifies the research aim. The next section presents market data and reviews literature on consumer perceptions of quality, safety, and sustainability, linking these to public health concerns such as obesity and diet-related illnesses. Subsequent sections analyze attitudes and motivations shaping purchasing decisions, integrating evidence from behavioral studies and market analysis. Finally, the conclusion synthesizes findings, emphasizing the dual role of organic food in promoting health and sustainability, and offering recommendations for policymakers and industry stakeholders.

GLOBAL MARKET TRENDS AND CONSUMER MOTIVATIONS FOR ORGANIC FOOD

The global organic food market has expanded rapidly in recent years. The global organic food and beverages sector was valued at approximately USD 231.5 billion in 2023 and is anticipated to expand significantly, reaching nearly USD 563.5 billion by 2030, with a compound annual growth rate of 13.6% during 2024-2030 (Grand View Research, 2024). Consumers are increasingly willing to pay premium prices for organic products, perceiving them as safer and of higher quality (Nandi et al., 2017).

The surge in organic food consumption is closely linked to rising incomes and heightened environmental awareness. Consumers increasingly recognize the ecological impact of conventional agriculture and prefer sustainable alternatives (Ornelas Herrera et al., 2025). Organic products are widely regarded as healthier and more environmentally responsible (Aschemann-Witzel et al., 2023). Concerns about pesticide residues and synthetic inputs in conventional farming further reinforce this perception (Zhou et al., 2025). Consistent evidence indicates that consumers view organic food as both safer and nutritionally superior to conventional alternatives (Aertsens et al., 2009). The rise in organic consumption intersects with pressing public health challenges. Although recent studies suggest obesity rates have stabilized, the condition continues to rank among the most critical public health issues (Goh & Tham, 2023; Ogden et al., 2014). The World Obesity Atlas 2023 projects that over 50% of the global population will be overweight or obese by 2035 (World Obesity Atlas, 2023).

Dietary habits, particularly frequent consumption of restaurant and fast food, are strongly linked to obesity due to higher calorie intake, saturated fats, added sugars, and artificial ingredients (Kruger et al., 2008). In contrast, organic food is positioned as the opposite dietary pattern: it emphasizes natural production methods, avoids synthetic additives, and is often perceived as healthier and more sustainable.

Table 1 presents the key consumer motivations for organic food consumption.

Table 1. Consumer motivations for organic food consumption

Motivation	Supporting studies	Description
Health and safety (perceived higher quality)	Aertsens et al. (2009)	Organic food seen as healthier and safer than conventional products
Environmental sustainability	Ornelas Herrera et al., (2025)	Preference for eco-friendly farming practices
Avoidance of pesticides and synthetic inputs	Zhou et al., (2025)	Concerns about conventional farming methods
Stabilization of obesity rates	Goh & Tham (2023); Ogden et al. (2014)	Obesity remains a significant public health issue
Eating outside food	Kruger et al. (2008)	Associated with higher calories, fats, sugars, and inorganic inputs

Nutrition and public health research identify multiple motivators behind healthier food choices. Earlier quantitative studies often relied on single-question measures, limiting insights into consumer decision-making, whereas more recent qualitative work has revealed deeper attitudes through interviews (Ashton et al., 2015; Verstraeten et al., 2014). Evidence suggests that healthy eating is frequently driven by intrinsic desires to improve physical and emotional well-being. For example, Jeong et al. (2018) found ‘feeling better and healthy’ to be the most significant motivator in the United States, with comparable trends reported in Europe (Michaelidou et al., 2012). Personal health consistently emerges as a central factor in dietary choices, influenced both by immediate concerns and by limited awareness of long-term benefits (Conner & Norman, 2021). Adults often select healthier foods to maintain well-being and protect health (Eikenberry & Smith, 2004; Jeong et al., 2018). At the organizational level, adequate nutrition is increasingly recognized as essential for reducing stress and supporting employee productivity and efficiency (Lukić & Nikolić, 2019).

Table 2 presents key health motivations behind consumer choices of organic food.

Table 2. Health motivations behind organic food choices

Motivation	Supporting studies	Key insight
Personal health concerns	Conner & Norman (2021)	Adults choose healthier foods to maintain health, though long-term benefits are not always well understood
Desire to maintain well-being	Eikenberry & Smith (2004); Jeong et al. (2018)	Health maintenance is a primary driver of healthier food selection
Intrinsic motivation	Mhlophe (2015)	Consumers link dietary choices directly to personal health outcomes
Increased sensitivity to nutrition	Kamenidou et al. (2019)	Modern consumers are more selective about nutritional choices

Beyond health, taste and quality also play a role, as studies show that flavor is an important motivator for organic purchases. Environmental and social responsibility further strengthen demand, with consumers appreciating farming practices that protect ecosystems and valuing transparency about where and by whom their food is produced. This growing demand not only supports the expansion of the organic food sector but also encourages farmers to adopt more sustainable cultivation methods, thereby contributing to the development of a healthier and more responsible food system (Wojciechowska-Solis et al., 2025).

CONSUMER BEHAVIOR TOWARD ORGANIC FOOD

In recent decades, consumers have increasingly shifted away from diets dominated by processed foods high in fats, sugars, and sodium, toward alternatives such as organic food that support digestive and immune health (Oliveira Faoro et al., 2024). Consumer behavior toward organic food is shaped by perceptions linking these products to health, safety, and sustainability. Studies show that buyers associate organic options with better nutrition, environmental protection, and higher quality, making these factors central to purchasing decisions. Positive perceptions of health and ecological benefits have fueled demand, with many consumers willing to pay premium prices despite higher costs (Shenoy et al., 2024).

Organic products are often perceived as healthier options because they are produced without synthetic chemicals, pesticides, or genetically modified inputs (Saleki et al., 2019). Growing consumer awareness of food safety issues, particularly concerning pesticide residues and genetic modification, has contributed to rising demand for organic alternatives (Miles & Frewer, 2001; Saleki et al., 2019). Consequently, the organic food market has expanded rapidly, driven by consumers’ desire for safer and more natural products (Bhattarai et al., 2013). Smith and Paladino (2010), drawing on estimates from the Rural Industries Research and Development Corporation, reported that the global organic industry was valued at approximately USD 33 billion and had been expanding at annual rates between 10–30%. These figures highlight the rapid global expansion of the organic industry.

Table 3 presents key perceptions regarding organic food as a healthier alternative.

Table 3. Organic food as a healthier alternative

Perception	Supporting studies	Implications
Free from synthetic chemicals/pesticides	Saleki et al. (2019); Miles & Frewer (2001)	Organic food perceived as safer and healthier
Avoidance of genetic modification	Saleki et al. (2019)	Consumers prefer natural, non-GM products
Food safety awareness	Saleki et al. (2019)	Rising concern about pesticide residues drives organic demand

By analyzing 21 primary studies published between 2006 and 2022, the findings suggest that regular intake of organic foods is consistently associated with reduced cardiometabolic risks, including lower prevalence of obesity, diabetes, hypertension, and hyperlipidemia. Clinical trials also confirm that individuals consuming organic diets experience significantly lower pesticide exposure, which may contribute to these health benefits (Poulia et al., 2025). In developing nations, organic food consumption is still emerging but shows promising growth. Consumers are increasingly aware of the nutritional and health benefits associated with organic products, which are perceived as more wholesome and safer (Rahman et al., 2024). A substantial body of literature demonstrates a positive correlation between health consciousness and consumer behavior, particularly in relation to organic food purchases (Rana & Paul, 2017). Health concerns consistently emerge as a key determinant influencing consumers' preference for organic over conventional products. Consumers increasingly associate dietary choices with measurable health outcomes, reinforcing the role of health motivations in shaping organic consumption patterns (Mhlophe, 2015). These motivations not only encourage the adoption of organic products but also indirectly influence purchasing intentions through heightened awareness of diet-related risks and benefits (Shenoy et al., 2024). Modern consumers are becoming more selective, paying closer attention to nutritional value and health implications of their food purchases (Kamenidou et al., 2019). Rising awareness has heightened sensitivity to health hazards, reinforcing the perception that organic products - free from pesticides and artificial chemicals are safer and more desirable (Roy et al., 2023). Health-conscious individuals are therefore more likely to prioritize organic food over conventional alternatives (Kamenidou et al., 2019). Environmentally conscious consumers often check labels carefully, willingly pay higher prices, and even accept lower product quality if it means supporting eco-friendly production. Their motivation is not driven by fashion but by genuine concern for ecological and social issues. As awareness of environmental challenges grows, consumer demand for organic food reflects a broader movement toward responsible consumption, signaling a cultural transition from purely self-oriented choices to behaviors rooted in sustainability and collective well-being (Wojciechowska-Solis et al., 2025).

CONCLUSION

The rising demand for organic food reflects a broader shift in consumer behavior, where health consciousness and environmental awareness increasingly shape choices. Organic products are valued for being free of synthetic chemicals and pesticides and are perceived as healthier and more sustainable alternatives to conventional food. This perception has fueled rapid global market growth, though adoption remains uneven across regions. At both individual and societal levels, motivations include personal health concerns, prevention of diet-related diseases, and growing awareness of food safety and sustainability. While the trend is more established in developed countries, emerging markets show strong potential as awareness spreads. Ultimately, organic food represents more than a market phenomenon: it is a public health and sustainability strategy. By aligning consumer demand with ecological practices, organic food addresses two global challenges - improving human health and reducing environmental impact. Future research should clarify long-term health outcomes of organic diets, examine cultural and socioeconomic differences in consumer behavior, and assess how price, labeling, and marketing influence accessibility and trust. Connecting ecological benefits with consumer awareness will be essential to shaping healthier lifestyles and more sustainable food systems worldwide.

REFERENCES

- Aertsens, J., Verbeke, W., Mondelaers, K., & Huylenbroeck, G.V. (2009). Personal Determinants of Organic Food Consumption: A Review. *British Food Journal*, 111(10), 1140-1167. <https://doi.org/10.1108/00070700910992961>
- Aschemann-Witzel, J., Asioli, D., Banovic, M., Perito, M.A., Peschel, A.O., & Stancu, V. (2023). Defining upcycled food: The dual role of upcycling in reducing food loss and waste. *Trends in Food Science & Technology*, 132, 132-137. <https://doi.org/10.1016/j.tifs.2023.01.001>
- Ashton, L.M., Hutchesson, M.J., Rollo, M.E., Morgan, P.J., Thompson, D.I., & Collins, C.E. (2015). Young adult males' motivators and perceived barriers towards eating healthily and being active: a qualitative study. *International Journal of Behavioral Nutrition and Physical Activity*, 12(1), 93-103. <https://doi.org/10.1186/s12966-015-0257-6>
- Ashaolu, T.J., & Ashaolu, J.O. (2020). Perspectives on the trends, challenges and benefits of green, smart and organic (GSO) foods. *International Journal of Gastronomy and Food Science*, 22, 100273. <https://doi.org/10.1016/j.ijgfs.2020.100273>
- Bhattarai, S., Lyne, M.C., & Martin, S.K. (2013). Assessing the performance of a supply chain for organic vegetables from a smallholder perspective. *Journal of Agribusiness in Developing and Emerging Economies*, 3(2), 101-118. <https://doi.org/10.1108/JADEE-12-2012-0031>
- Conner, M., & Norman, P. (2021). Predicting long-term healthy eating behaviour: understanding the role of cognitive and affective attitudes. *Psychology & Health*, 36(10), 1165-1181. <https://doi.org/10.1080/08870446.2020.1832675>
- Eikenberry, N., & Smith, C. (2004). Healthful eating: perceptions, motivations, barriers, and promoters in low-income Minnesota communities. *Journal of the American Dietetic Association*, 104(7), 1158-1161. <https://doi.org/10.1016/j.jada.2004.04.023>
- Goh, G.B.B., & Tham, K.W. (2023). Combating obesity: a change in perspectives. *Singapore Medical Journal*, 64(3), 153-154. <https://doi.org/10.4103/singaporemedj.SMJ-2023-043>
- Grand View Research. (2024). Organic food and beverages market (2024-2030). Size, Share & Trends Analysis Report by Product (Organic Food, Organic Beverages), by Distribution Channel (Convenience Stores, Specialty Stores), by Region, and Segment Forecasts. Retrieved from <https://www.grandviewresearch.com/industry-analysis/organic-foods-beverages-market>
- Jeong, E., Jang, S.S., Behnke, C., Anderson, J., & Day, J. (2018). A scale for restaurant customers' healthy menu choices: Individual and environmental factors. *International Journal of Contemporary Hospitality Management*, 31(10), 217-246. <https://doi.org/10.1108/IJCHM-06-2017-0377>
- Kamenidou, I.C., Mamalis, S.A., Pavlidis, S., & Bara, E.Z.G. (2019). Segmenting the generation Z cohort university students based on sustainable food consumption behavior: A preliminary study. *Sustainability*, 11(3), 837. <https://doi.org/10.3390/su11030837>
- Kruger, J., Blanck, H.M., & Gillespie, C. (2008). Dietary practices, dining out behavior, and physical activity correlates of weight loss maintenance. *Preventing Chronic Disease*, 5(1), 1-14.
- Latinović, B., Ostojić, B., & Bugarčić, M. (2023). Analysis of attitudes towards social-economic and marketing factors on the growth of organic production. *Economic of Agriculture*, 70(3), 725-735. <https://doi.org/10.59267/ekoPolj2303725L>
- Lukić, J., & Lazarević, S. (2019). A holistic approach to workplace stress management. *School of Business*, 1, 130-144. <https://doi.org/10.5937/skolbiz1-21872>
- Michaelidou, N., Christodoulides, G., & Torova, K. (2012). Determinants of healthy eating: a crossnational study on motives and barriers. *International Journal of Consumer Studies*, 36(1), 17-22. <https://doi.org/10.1111/j.1470-6431.2011.01031.x>
- Miles, S., & Frewer, L.J. (2001). Investigating specific concerns about different food hazards. *Food Quality and Preference*, 12(1), 47-61. [https://doi.org/10.1016/S0950-3293\(00\)00029-X](https://doi.org/10.1016/S0950-3293(00)00029-X)
- Mhlophe, J.B. (2015). Antecedents of consumer purchase intentions towards organic food produces: a case study of the Johannesburg municipality (Doctoral dissertation, University of the Witwatersrand, Faculty of Commerce, Law and Management, School of Economic and Business Sciences).

- Nandi, R., Bokelmann, W., Gowdru, N.V., & Dias, G. (2017). Factors influencing consumers' willingness to pay for organic fruits and vegetables: Empirical evidence from a consumer survey in India. *Journal of Food Products Marketing*, 23(4), 430-451. <https://doi.org/10.1080/10454446.2015.1048018>
- Ogden, C.L., Carroll, M.D., Kit, B.K., & Flegal, K.M. (2014). Prevalence of childhood and adult obesity in the United States, 2011-2012. *Journal of the American Medical Association*, 311(8), 806-814. <https://doi.org/10.1001/jama.2014.732>
- Oliveira Faoro, D.T., Artuzo, F.D., Borges, J.A.R., Foguesatto, C.R., Dewes, H., & Talamini, E. (2024). Are organics more nutritious than conventional foods? A comprehensive systematic review. *Heliyon*, 10(7), e28288. <https://doi.org/10.1016/j.heliyon.2024.e28288>
- Ornelas Herrera, S.I., Baba, Y., Kallas, Z., Meers, E., Michels, E., Hajdu, Z., & Spicnagel, A.M. (2025). The role of environmental attitudes and consumption patterns in consumers' preferences for sustainable food from circular farming system: a six EU case studies. *Agricultural and Food Economics*, 13(1), 7. <https://doi.org/10.1186/s40100-025-00350-0>
- Ostojić, B., Petrović, V., & Despotović, D. (2025). Consumer perceptions of geographical indications, branding, and intellectual property in the agricultural sector. *Economic of Agriculture*, 72(4), 1359-1374. <https://doi.org/10.59267/ekoPolj25041359O>
- Poulia, K.A., Bakaloudi, D.R., Alevizou, M., Papakonstantinou, E., Zampelas, A., & Chourdakis, M. (2025). Impact of organic foods on chronic diseases and health perception: a systematic review of the evidence. *European Journal of Clinical Nutrition*, 79, 90-103. <https://doi.org/10.1038/s41430-024-01505-w>
- Rahman, A., Baharlouei, P., Koh, E.H.Y., Pirvu, D.G., Rehmani, R., Arcos, M., & Puri, S. (2024). A Comprehensive Analysis of Organic Food: Evaluating Nutritional Value and Impact on Human Health. *Foods*, 13(2), 208. <https://doi.org/10.3390/foods13020208>
- Rana, J., & Paul, J. (2017). Consumer behavior and purchase intention for organic food: A review and research agenda. *Journal of Retailing and Consumer Services*, 38, 157-165. <https://doi.org/10.1016/j.jretconser.2017.06.004>
- Roy, A., Ghosh, A., & Vashisht, D. (2023). The consumer perception and purchasing attitude towards organic food: A critical review. *Nutrition & Food Science*, 53(3), 578-599. <https://doi.org/10.1108/NFS-04-2022-0130>
- Saleki, R., Quoquab, F., & Mohammad, J. (2019). What drives Malaysian consumers' organic food purchase intention? The role of moral norm, self-identity, environmental concern and price consciousness. *Journal of Agribusiness in Developing and Emerging Economies*, 9(5), 584-603. <https://doi.org/10.1108/JADEE-02-2019-0018>
- Shenoy, K.R., Ranjith, V.K., Nayak, S., & Anirvinna, C. (2024). Conceptualizing organic food consumption: a consumer motive perspective. *Cogent Social Sciences*, 10(1). <https://doi.org/10.1080/23311886.2024.2338864>
- Smith, S., & Paladino, A. (2010). Eating clean and green? Investigating consumer motivations towards the purchase of organic food. *Australasian Marketing Journal (AMJ)*, 18(2), 93-104. <https://doi.org/10.1016/j.ausmj.2010.01.001>
- Verstraeten, R., Van Royen, K., Ochoa-Avilés, A., Penafiel, D., Holdsworth, M., Donoso, S., Maes, L., & Kolsteren, P. (2014). A conceptual framework for healthy eating behavior in ecuadorian adolescents: a qualitative study. *PloS One*, 9(1), e87183. <https://doi.org/10.1371/journal.pone.0087183>
- World Obesity Atlas (2023). World Obesity. Retrieved from <https://www.worldobesity.org/resources/resource-library/world-obesity-atlas-2023> (14 May 2026)
- Wojciechowska-Solis, J., Śmiglak-Krajewska, M., Smoluk-Sikorska, J., Malinowski, M., Krnáčová, P., Jarossová, M.A., & Kis, G.G. (2025). Factors Determining Buying Behavior on the Organic Food Market in the Visegrad Group Countries-Using Canonical Correlation Analysis. *Sustainability*, 17(2), 672. <https://doi.org/10.3390/su17020672>
- Zhou, W., Li, M., Achal, V. (2025). A comprehensive review on environmental and human health impacts of chemical pesticide usage. *Emerging Contaminants*, 11(1), 100410. <https://doi.org/10.1016/j.emcon.2024.100410>

Sustainable agricultural production as a determinant of agrotourism development in rural areas: An empirical study in the Republic of Srpska

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Abstract

Agrotourism is increasingly recognized in contemporary research as an important instrument for the sustainable development of rural areas, as it contributes to the diversification of economic activities, the preservation of cultural heritage, and the improvement of the quality of life in rural communities. The aim of this research was to examine the relationship between sustainable agricultural production and the development of agrotourism. The study was conducted on a sample of 419 respondents using a five-point Likert scale, while descriptive statistics and linear regression methods were applied for data analysis. The results indicate a high level of agreement among respondents with statements related to the importance of agrotourism for the economic development of rural areas ($M = 4.47$) and the preservation of traditional culture ($M = 4.33$). The results of the Analysis of Variance ANOVA confirm the statistical significance of the regression model as a whole [$F(1, 417) = 667.8680, p < 0.0001$]. Linear regression analysis shows that sustainable agricultural production has a statistically significant positive impact on the development of agrotourism ($\beta = 0.784615; p < 0.0001$), with the model explaining 61.56% of the variance of the dependent variable ($R^2 = 0.615621$). The obtained results confirm that the integration of sustainable agricultural practices and tourism activities represents an important factor in the development of the rural economy. The proposed hypothesis (H1), stating that sustainable agricultural production has a statistically significant positive relationship with agrotourism development, was confirmed.

Keywords: agrotourism, sustainable agricultural production, rural development, diversification of the rural economy, sustainable tourism

INTRODUCTION

„Growing interdependence among economic and social systems has significantly affected rural development and agricultural production, creating new development challenges shaped largely by climate change and evolving consumer preferences“ (Akimowicz et al., 2023). At the same time, „rural regions contain valuable resources whose importance is increasingly recognized in contemporary society and the economy, including farmland, natural assets, cultural heritage, and traditional methods of production“ (Miroshnichenko et al., 2024). „The combination of these elements has encouraged the development of agrotourism, a tourism model connected to agricultural activities and rural settings that can contribute to economic growth while simultaneously supporting the preservation of rural communities and traditions“ (Ammirato et al., 2020). An analysis of the economic effects of tourism reveals the following impacts of tourism on agriculture, including: increased agricultural production and improved market placement of agricultural products, impact on employment and the living standards of the population, stopping emigration from certain villages and municipalities due to lack of employment

opportunities, encouragement of natural population growth, development of underdeveloped and less developed areas, and higher earnings for employees.

Also, the effects of agrotourism on the economic development of a country can be observed through tourism-related consumption. „Various domestic and international studies indicate that revenue generated through tourism services provided to foreign tourists circulates within the national economy and contributes to broader economic development effects“ (Sinclair, 1998). In this context, „agrotourism is increasingly viewed as an important factor in the sustainable development of rural areas because it contributes to the diversification of farmers' income sources, preservation of cultural heritage, and strengthening of environmental awareness“ (Iannucci et al., 2022). „Furthermore, recent research points to a growing interest in examining the relationship between agriculture and tourism, particularly in terms of cultural heritage preservation and environmental responsibility“ (Yasin & Bacsı, 2025).

Although rural tourism and agrotourism are closely related and complementary concepts, important conceptual differences exist between them. Rural tourism encompasses a broad range of activities taking place in rural areas, including those related to local traditions, cultural content, and heritage exploration. „Agrotourism, however, is more specifically connected to agricultural activities and direct interaction between visitors and farms“ (Gajić et al., 2024). „Through participation in activities such as fruit harvesting or the preparation of traditional local products, visitors gain an authentic experience of rural life“ (Lane & Kastenholz, 2015).

The aim of this research is to analyze the relationship between sustainable agricultural production and the development of agrotourism in the Republic of Srpska, based on the views of agricultural producers, in order to examine the hypothesis of a statistically significant relationship between these variables, presented in the Methods and Materials section of the paper.

Agrotourism as an Instrument of Sustainable Rural Development

In contemporary literature, „agrotourism is increasingly viewed as an important instrument for the development of rural areas, as it generates various social and economic effects“ (Yasin & Bacsı, 2025). Unlike traditional agriculture, which is often exposed to seasonal fluctuations and climatic risks, tourism-related activities provide more stable and diverse sources of income. Rural households that develop offerings such as accommodation services, gastronomy, educational programs, or cultural events can reduce their dependence on the sale of agricultural products and enhance the economic resilience of farms.

According to the definition of the European Commission, „rural tourism encompasses all tourism activities that take place in rural areas“ (Ana, 2017). Within this framework, several levels of conceptual determination can be distinguished. Rural tourism represents the broadest concept and includes various forms such as wine, gastronomic, hunting, fishing, religious, cultural, and adventure tourism, as well as activities in national parks and areas. „Rural tourism includes and is linked to the rural environment and its immediate surroundings, including excursion sites, rural holiday homes, family hotels, ethno-villages, and traditional events“, argue (Drinić & Rokvić-Knežić, 2021). Within that concept, „agrotourism represents more specific forms that involve tourism activities organized on agricultural farms, where visitors are offered services such as accommodation, food, and various activities related to staying in a rural environment“ (Rover, 2018).

„Agrotourism is considered a significant segment of rural tourism“ (Popescu, 2010), as, in combination with other forms such as ecotourism, cultural tourism, or nature-based activities, it contributes to the diversity and attractiveness of the overall tourism offer. Its development intensified particularly at the end of the nineteenth century, when it was recognized as an opportunity to supplement the income of agricultural farms. In this way, „the economic sustainability of farms is extended, and the likelihood that families will remain in rural areas and continue their production activities is increased“ (Angelkova, 2012).

In modern research, „agrotourism is often perceived as an instrument of sustainable development for rural communities, because it contributes to income diversification, preservation of cultural heritage, strengthening of environmental awareness, and the development of social capital“ (Demirović-Bajrami et al., 2020). „The integration of agricultural production and tourism activities enables the creation of

additional value for households and supports the long-term economic development of rural areas” (Zvavahera & Chigora, 2023).

Within the framework of the sustainability concept, (Yusuf & Wulandari, 2023) point to „the need to develop efficient agrotourism models that integrate ecological principles, the role of local stakeholders, and adequate institutional support”. Particular emphasis is placed on expanding the tourism offer, applying modern marketing approaches, developing partnerships, and actively involving the local community in tourism-related activities.

According to (Pejanović & Vujović, 2008), „agrotourism represents a strong link between tourism and agriculture, contributing to the sustainable development of the agro-complex”. Visitors to rural destinations most often seek authentic experiences, such as tasting local products, participating in everyday farm activities, or becoming familiar with traditional way of life.

In addition to its economic effects, this activity contributes to strengthening social relations in rural areas. „Rural residents often perceive tourism as an opportunity to preserve cultural identity and enhance local development”, note (Demirović-Bajrami et al., 2020). The benefits of tourism are not only related to the financial aspect, but also include improvements in infrastructure, the creation of new jobs, and greater community participation in social activities. Agricultural productivity acquires a broader meaning, as it implies the ability of rural areas to generate additional value from available resources through tourism.

Agrotourism can also be viewed „as a form of diversification of farm activities” (Wu et al., 2024). In addition to primary agricultural production, farmers generate additional income through services, educational programs, and experiential activities for visitors. Unlike the traditional expansion of the product range, the inclusion of tourism activities enables opportunities for direct product sales, the organization of tastings, and various events, thereby reducing dependence on intermediaries and strengthening the relationship with final consumers.

Improving productivity in agriculture is associated with the diversification of production, the application of modern and sustainable practices, as well as greater adaptability to climate change. Higher standards of food quality and safety contribute to product competitiveness and facilitate access to higher-value markets. According to (Williams Forson & Counihan, 2012), „the inclusion of local products in the tourism offer increases their market value, as they become part of the authentic experience of the destination”.

The ecological aspect of agrotourism is reflected in the preservation of landscapes, the use of indigenous crop varieties, the application of traditional production methods, and the development of organic production. Such an approach contributes to the protection of natural resources while simultaneously creating an attractive offer for visitors. Tourists’ search for authentic and natural environments encourages producers to invest in high-quality and sustainable production models. The need for innovation and adaptation to climate change is also emphasized, as agrotourism connects the economic, ecological, and social dimensions of rural development.

Agrotourism also contributes to strengthening the market position of agricultural products, because tourists’ purchases of local specialties directly support producers. At the same time, the presentation of traditional dishes, processed products, and handicrafts helps preserve the cultural identity of rural communities and develop an attractive tourism offer. „The successful development of agrotourism requires a strong connection between agriculture and tourism, with gastronomy representing an important element of differentiation and competitiveness of rural destinations”, emphasize (Pejanović & Vujović, 2008). The practical implementation of this concept involves the promotion of local products, direct communication between producers and tourists, the adaptation of offerings to visitors’ preferences, and the preservation of tradition through gastronomic and educational activities. „Agrotourism contributes to the creation of additional value for households and strengthens the rural economy”, argue (Zvavahera & Chigora, 2023).

Gastronomic products play a particularly important role in the development of rural tourism. Local culinary tradition is a key component of the tourist experience in rural areas. As consumers increasingly value organic production and make purchasing decisions based on trust in producers, rural tourism creates a unique advantage by enabling direct contact between producers and consumers. „Visitors have

the opportunity to learn about the production process and the families behind the products, thereby building trust that is difficult to achieve in mass markets”, note (Dašić et al., 2019).

Local communities often perceive tourism not only as an economic mechanism but also as a means of improving quality of life and strengthening social cohesion. Within such a model, farm products are not just goods but part of an authentic experience offered to tourists, so the competitive advantage stems from: quality, trust, and the overall experience. The integration of sustainable agricultural production and tourism activities enables increased consumer trust through direct communication, the development of gastronomic offerings based on local products, the strengthening of the market position of farms, and the preservation of cultural identity through the presentation of traditional food. „Sustainable production, in synergy with tourism, evolves into an important instrument for the comprehensive development of rural areas”, according to (Ivona, 2021).

Development of the agrotourism offer in rural areas of the Republic of Srpska

In the Republic of Srpska, „agrotourism represents a key instrument for improving the market position of agriculture”, argue (Mrdalj & Todić, 2012). Through the diversification of offerings, including the promotion of local specialties, the establishment of direct contact between tourists and producers, and the differentiation of services according to visitor needs, agricultural farms generate significant additional income and strengthen their competitiveness. Local gastronomic products play a particularly important role in this process. When a tourist observes the production process, meets the host, and senses the scent of tradition, they cease to be just a consumer and become a participant in heritage preservation. Agrotourism not only generates economic value but also functions as a custodian of tradition, gastronomy, and educational activities that enrich the rural tourism offer. „The importance of agrotourism in the Republic of Srpska has been formally recognized as a development opportunity through several key strategic and legal documents”, note (Berjan et al., 2014).

The strategic framework of the Republic of Srpska has acknowledged these potentials. By adopting the Tourism Development Strategy for the period 2021-2027, the foundations were established for improving the legal and regulatory framework, creating a favorable investment environment for tourism infrastructure, and developing new tourism products, with particular emphasis on rural tourism and nature-based experiences. The adoption of the Agriculture and Rural Development Strategy (2021-2027), although primarily agricultural in focus, is also crucial for agrotourism as it addresses income diversification in rural areas. By revitalizing villages, it encourages young people to remain in rural communities by launching tourism services alongside primary agricultural production. Additionally, the Ministry of Agriculture, Forestry, and Water Management annually issues regulations for incentives that include investments in rural tourism.

It is important to note that the development of agrotourism is not possible without the active involvement of local communities. In the rural areas of the Republic of Srpska, residents’ perceptions of the economic, social, and environmental benefits of tourism can directly influence the community’s willingness to support sustainable tourism initiatives. „Rural tourism development cannot be viewed as a top-down project, but rather it requires the active participation of residents who possess the resources and traditions”, argue (Demirović-Bajrami et al., 2020). Given that rural areas of the Republic of Srpska have a significant number of small farms with land, forests, meadows, and orchards, there is potential to valorize these resources on the market through agrotourism.

Visitors, particularly from urban areas, increasingly seek authentic experiences, such as staying in natural settings, tasting local foods, participating in agricultural activities, and learning about tradition. Agrotourism can be sustainable because it integrates economic benefits (additional farm income), social benefits (community preservation, cultural identity, and intergenerational solidarity), and environmental benefits (preservation of landscape, traditional varieties and natural environment).

MATERIAL AND METHODS

The research is based on a qualitative analysis of relevant domestic and international scientific literature in the fields of sustainable agricultural production and agrotourism, as well as scientific and professional publications with related topics. The method of content analysis of available secondary sources was applied, including a review of official documents such as: national strategies, regulations, and professional guidelines. The quantitative part of the research was conducted in a combined field and online format on the territory of the Republic of Srpska, with the help of agricultural and tourism associations, anonymously, in the period from January 1, 2026 to February 15, 2026.

For the purposes of the quantitative part of the research, on a sample of 419 randomly selected respondents engaged in agricultural production from the territory of the Republic of Srpska, a five-point Likert scale was applied. The respondents assessed statements related to the variables Agrotourism and Sustainable Agricultural Production (Table 1).

Hypothesis H₁, sustainable agricultural production is statistically significant and has a positive effect on the development of agrotourism.

The results of the descriptive statistics indicate a high level of agreement among respondents regarding statements related to the significance of Agrotourism and Sustainable Agricultural Production. Mean response values (Mean) range from 3.95 to 4.47, indicating that respondents largely believe that the development of agrotourism contributes to the economic development of rural areas, enhances the attractiveness of rural destinations for domestic and international tourists, and supports the preservation of traditional culture, customs, and rural ways of life.

Table 1. Descriptive Statistics Results of Variables

Variable Agrotourism	Mean	SD
The development of agrotourism significantly contributes to the economic development of rural areas.	4.47	0.59
Agrotourism increases the attractiveness of rural destinations for both domestic and international tourists.	4.36	0.62
The development of agrotourism contributes to the preservation of traditional culture, customs, and ways of life in rural communities.	4.34	0.54
Variable Sustainable agricultural production	Mean	SD
The application of the principles of sustainable agricultural production contributes to the preservation of the environment and natural resources.	3.96	0.52
Sustainable agricultural production contributes to improving food quality and safety.	4.16	0.55
The development of sustainable agricultural production creates favorable conditions for the development of agrotourism in rural areas.	4.34	0.55

Source: Author's calculation

The results also show that respondents recognize the importance of sustainable agricultural production, particularly in the context of environmental and natural resource conservation, as well as improvements in food quality and safety. Relatively low standard deviation (SD) values from 0.52 to 0.62 indicate a high degree of homogeneity in respondent's attitudes. It can be concluded that there is a positive relationship between the perception of sustainable agricultural production and agrotourism in rural areas.

RESULTS AND DISCUSSION

The results of the linear regression analysis Summary of Fit (Table 2) indicate the presence of a statistically significant and strong relationship between sustainable agricultural production and the development of agrotourism. The model analysis shows that the coefficient of determination ($R^2=0.615621$) suggests that approximately 61.56% of the variance in the dependent variable, agrotourism, can be explained by the

influence of the independent variable, namely sustainable agricultural production. Moreover, the adjusted coefficient of determination ($\text{Adjusted } R^2=0.6147$) is almost identical, confirming the stability of the model and indicating that the inclusion of this variable significantly contributes to explaining variability in agrotourism development. The relatively low value of the standard error of the estimate (Root Mean Square Error=0.259239) further confirms the model's precision and indicates that the model's predictions exhibit relatively small deviations from the observed empirical values.

Table 2. Summary of Fit

RSquare	0.615621
RSquare Adj	0.6147
Root Mean Square Error	0.259239
Mean of Response	4.154336
Observations (or Sum Wgts)	419

Source: Author's calculation

The results of the Analysis of Variance ANOVA (Table 3) confirm the statistical significance of the regression model as a whole. The value [$F(1,417)=667.8680$, $p<0.0001$] clearly indicates that the model is highly reliable and that a significant linear relationship exists between the analyzed variables. The high F-statistic demonstrates that the variance explained by the model is substantially greater than the unexplained variance, or error, thereby confirming the adequacy of the regression model for interpreting the relationship between sustainable agricultural production and agrotourism.

Table 3. Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	44.884043	44.8840	667.8680
Error	417	28.024470	0.0672	Prob > F
C. Total	418	72.908512		<0.0001

Source: Author's calculation

The Parameter Estimates analysis (Table 4) further confirms the significant positive impact of sustainable agricultural production on the development of agrotourism. The regression coefficient is (0.7167564) and is highly statistically significant ($t=25.84$; $p<0.0001$), indicating that an increase in the level of sustainable agricultural production leads to a proportional increase in agrotourism development. In other words, the improvement of sustainable agricultural practices such as the rational use of natural resources, biodiversity conservation, and the application of environmentally friendly technologies can substantially contribute to the development of tourism activities in rural areas.

The standardized beta coefficient (Std Beta=0.784615) confirms the strong effect of this variable, indicating that sustainable agriculture is one of the key factors influencing agrotourism development. Additionally, the variance inflation factor ($VIF=1$) indicates the absence of multicollinearity, confirming that the parameter estimates are stable and statistically reliable.

The model intercept 1.0084764 is also statistically significant ($p<0.0001$), implying that even at minimal values of the independent variable, there is a baseline level of agrotourism development, which may be attributable to other factors such as natural attractions, cultural heritage, or institutional support for rural tourism development.

Based on these results, Hypothesis H_1 , stating that sustainable agricultural production has a statistically significant positive effect on the development of agrotourism, is supported.

Table 4. Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>t	Std Beta	VIF
Intercept	1.0084764	0.122386	8.24	<0.0001	0	.
Sustainable Agricultural Production	0.7167564	0.027735	25.84	<0.0001	0.784615	1

Source: Author's calculation

The findings support theoretical assumptions that sustainable agricultural systems contribute to the diversification of the rural economy and the creation of added value through tourism activities. The integration of sustainable agricultural practices with tourism offerings can contribute to enhancing the competitiveness of rural destinations, preserving the environment, and promoting the socio-economic development of rural communities.

Similar findings are supported by numerous international studies. Research conducted by (Barbieri & Mshenga, 2008) indicates that „the diversification of agricultural activities through agrotourism contributes not only to increased economic benefits for agricultural farms but also to the improvement of sustainable resource management“. Sustainable agricultural practices enhance the authenticity and attractiveness of the tourism offer in rural areas. Lane and Kastenholz (2015) emphasize that „the development of rural tourism depends on the preservation of natural resources, local culture, and sustainable agricultural systems“. Sustainable agriculture provides the foundation for creating authentic tourism products that appeal to contemporary tourists interested in ecologically responsible and genuine destinations.

Phillip et al. (2010) highlight that „agrotourism represents an important instrument for diversifying the rural economy, where sustainable food production and the preservation of traditional agricultural practices play a key role in shaping the tourism offer“. Tourists increasingly seek experiences connected to local food production, organic farming, and authentic rural environments.

The results obtained in this study corroborate these theoretical and empirical findings, indicating that the improvement of sustainable agricultural production can significantly contribute to the development of agrotourism. The integration of agricultural production, local gastronomy, and tourism activities generates additional value for rural areas, enhances the competitiveness of tourist destinations, and supports sustainable economic development. These research outcomes carry important practical implications for agrarian and tourism policy makers, highlighting the need to promote sustainable agricultural practices, support the diversification of activities on farms, and strengthen the integration between agriculture and tourism.

CONCLUSION

The development of agrotourism in the rural areas of the Republic of Srpska represents an important instrument for enhancing the market position of agriculture, strengthening the economic resilience of villages, and preserving cultural and natural identity. Agrotourism provides agricultural farms with the opportunity to diversify their income sources by linking primary production with a variety of tourism-related services, including accommodation, gastronomic offerings, and educational or cultural activities. This approach contributes to the creation of added value within local communities, encourages the preservation of traditional production methods, and attracts visitors interested in an authentic rural experience. Direct communication between producers and consumers strengthens trust in the quality of local products, facilitates adaptation to market demands, and enhances the competitive position of farms. The introduction of complementary products and services such as locally processed food products, souvenirs, or thematic workshops further expands the tourism offer and enables the sale of products in targeted niche markets. The results of the conducted study confirm a significant relationship between sustainable agricultural production and the development of agrotourism in rural areas. Descriptive statistics indicate a high level of agreement among respondents regarding the importance of agrotourism for the economic development of villages, the enhancement of rural destination attractiveness, and the preservation of traditional culture. Simultaneously, respondents recognize the importance of sustainable agricultural production for environmental conservation, the improvement of food quality, and the creation

of favorable conditions for the development of tourism activities in rural communities. ANOVA results confirmed the model's significance [$F(1, 417)=667.87$; $p<0.001$], identifying sustainable agricultural production as a significant predictor of agrotourism development $\beta=0.784615$ and $p<0.0001$. The coefficient of determination 0.615621 indicates that the model explains 61.56% of the dependent variable's variance. Based on results, the proposed hypothesis H_1 that sustainable agricultural production has a statistically significant positive impact on agrotourism development is confirmed. In the broader context of rural development, the study's findings indicate that the integration of sustainable agricultural practices with tourism activities represents an important instrument for diversifying the rural economy. This approach contributes to enhancing the competitiveness of rural destinations, preserving natural resources, promoting local products, and strengthening the socio-economic development of rural communities. In the future, greater integration of digital technologies into agrotourism development is expected, including online promotion, direct internet sales, virtual tours, and interactive educational programs. These innovations enable rural households to access a wider audience, increase revenue, and develop sustainable business models. The importance of ecologically and climate-responsible production will continue to grow, with particular emphasis on biodiversity conservation, the use of renewable energy sources, and the reduction of negative environmental impacts. Future agrotourism models should take into account local cultural, economic, and ecological specificities to ensure long-term sustainability and maximize benefits for local communities. The conducted research has confirmed that the development of agrotourism in the Republic of Srpska is not only a complementary activity, but a strategic direction to strengthen the economic resilience of rural areas and preserve their cultural identity. The results of the research indicate that the modernization of agricultural holdings through income diversification directly relies on their ability to integrate primary production with tourism services. Sustainable agricultural production is a fundamental pillar of the development of agrotourism in the Republic of Srpska. Farms that apply the principles of environmental protection and high food quality standards have significantly greater potential for attracting tourists and achieving market competitiveness.

REFERENCES

- Akimowicz, M., Weeden, S., & Gibson, R. (2023). Building bridges across the plurality of rural development research. *Annals of Regional Science*, 71, 1-8. <https://doi.org/10.1007/s00168-023-01224-3>.
- Ammirato, S., Felicetti, A., Raso, C., Pansera, B., & Violi, A. (2020). Agritourism and Sustainability: What We Can Learn from a Systematic Literature Review. *Sustainability*, 12(22), 9575. <https://doi.org/10.3390/su12229575>
- Ana, M. I. (2017). Ecotourism, agro-tourism and rural tourism in the European Union. *Cactus Tourism Journal*, 15(2), 6-14.
- Angelkova, T. (2012). Uloga specifičnih vidova turizma u revitalizaciji pograničnih područja između Srbije i Makedonije. *Doktorska disertacija*. Univerzitet Singidunum, Beograd. [The role of specific forms of tourism in the revitalization of border areas between Serbia and Macedonia. *Doctoral dissertation*. Singidunum University, Belgrade].
- Barbieri, C., & Mshenga, P. M. (2008). The role of the firm and owner characteristics on the performance of agrotourism farms. *Sociologia Ruralis*, 48(2), 166-183. <https://doi.org/10.1111/j.1467-9523.2008.00450.x>
- Berjan, S., El Bilali, H., Radovic, G., Sorajić, B., Driouech, N., & Radosavac, A. (2014). Rural tourism in the Republika Srpska: Political framework and institutional environment. *Turkish Journal of Agricultural and Natural Sciences*, 1(2), 1805-1811. <https://izlik.org/JA28RB95PY>
- Dašić, G., Radosavac, A., Knežević, D., & Đervida, R. (2019). Preferences of customers and improvement of production and sales of organic products in Serbia. *Economics of Agriculture*, 66(1), 71-86. <https://doi.org/10.5937/ekoPolj1901127D>
- Demirović-Bajrami, D., Radosavac, A., Cimbaljević, M., Tretiakova, T. N., & Syromiatnikova, Y. A. (2020). Determinants of Residents' Support for Sustainable Tourism Development: Implications for Rural Communities. *Sustainability*, 12(22), 9438. <https://doi.org/10.3390/su12229438>

- Drinić, Lj., & Rokvić-Knežić, G. (2021). *Žensko preduzetništvo u razvoju ruralnih područja*. Univerzitet u Banjoj Luci, Poljoprivredni fakultet, [Women's Entrepreneurship in the Development of Rural Areas. University of Banja Luka, Faculty of Agriculture], 113-119.
- Gajić, T., Petrović, M. D., Blešić, I., Radovanović, M. M., Spasojević, A., Sekulić, D., & Dubover, D. A. (2024). The Contribution of the Farm to Table Concept to the Sustainable Development of Agrotourism Homesteads. *Agriculture*, 14(8), 1314. <https://doi.org/10.3390/agriculture14081314>
- Iannucci, G., Martellozzo, F., & Randelli, F. (2022). Sustainable development of rural areas: A dynamic model in between tourism exploitation and landscape decline. *Journal of Evolutionary Economics* 32, 991-1016. <https://doi.org/10.1007/s00191-022-00785-4>
- Ivona, A. (2021). Sustainability of rural tourism and promotion of local development. *Sustainability*, 13(16), 8854. <https://doi.org/10.3390/su13168854>
- Lane, B., & Kastenholz, E. (2015). Rural tourism: the evolution of practice and research approaches—towards a new generation concept? *Journal of Sustainable Tourism*, 23(8-9), 1133-1156. <https://doi.org/10.1080/09669582.2015.1083997>
- Miroshnichenko, I., Doroshenko, O., Tereshina, M., Rakachev, V., Morozova, E., Golub, M., & Shpiro, L. (2024). Analysing Rural Development Models Based on Intangible Assets and Socio-Economic Development. *Sustainability*, 16(23), 10613. <https://doi.org/10.3390/su162310613>
- Mrdalj, V., & Todić, D. (2012). *Agriculture and the role of cooperatives in rural tourism development in the Republic of Srpska*. Third International Scientific Symposium Agrosym Jahorina. Proceedings, 718-722. 10.7251/AGSY1203718M
- Pejanović, R. & Vujović, S. (2008). Ruralni razvoj i agroturizam. *Agroekonomika* [Rural development and agrotourism, *Agroeconomics*], 37-38, 5-14.
- Phillip, S., Hunter, C., & Blackstock, K. (2010). A typology for defining agrotourism. *Tourism Management*, 31(6), 754-758. <https://doi.org/10.1016/j.tourman.2009.08.001>
- Popesku, J. (2010). *Turizam posebnih interesovanja - Selektivni oblici turizma*, Beograd. [Special Interest Tourism - Selective Forms of Tourism, Belgrade].
- Rover, S. (2018). Planiranje ruralnog turizma, integralni i održivi pristup. *Master rad*. Univerzitet u Banjoj Luci, Poljoprivredni fakultet. [Rural tourism planning, an integrated and sustainable approach. *Master's thesis*. University of Banja Luka, Faculty of Agriculture].
- Sinclair, M. T. (1998). Tourism and economic development: A survey. *The Journal of Development Studies*, 34(5), 1–51. <https://doi.org/10.1080/00220389808422535>
- Vlada Republike Srpske i Ministarstvo trgovine i turizma (2021). *Strategija razvoja turizma Republike Srpske za period (2021–2027)*. [Government of Republika Srpska and Ministry of Trade and Tourism (2021). *Tourism Development Strategy of Republika Srpska for the period (2021–2027)*].
- Vlada Republike Srpske, Ministarstvo poljoprivrede, šumarstva i vodoprivrede Republike Srpske (2021). *Strategija razvoja poljoprivrede i ruralnih područja (2021–2027)*. [Government of the Republika Srpska, Ministry of Agriculture, Forestry and Water Management of the Republika Srpska (2021). *Strategy for the Development of Agriculture and Rural Areas (2021–2027)*].
- Yasin, A. S., & Bacsı, Z. (2025). Agrotourism and rural development: A global bibliometric analysis of the state of research, limitations, and future directions. *Agriculture*, 15(8), 866. <https://doi.org/10.3390/agriculture15080866>
- Williams Forson, P., & Counihan, C. (2012). Taking food public: Redefining foodways in a changing world (1st ed.). *Routledge*. <https://doi.org/10.4324/9781315881065>
- Wu, T. C. E., Chen, C. P., Hsu, A. Y. C., & Wall, G. (2024). Farm diversification through agrotourism: innovation synergies. *International Food and Agribusiness Management Review*, 27(3), 509-525. <https://doi.org/10.22434/ifamr2022.0167>
- Zvavahera, P., & Chigora, F. (2023). Agrotourism: a source for socio-economic transformation in developing economies. *Qeios*, 1, 1-16. <https://doi.org/10.32388/DXTYIG>

Bridging the sustainability data gap in the Western Balkans: The 'ESG-Admin' model applied to Bosnia and Herzegovina

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Abstract

Strict EU sustainability reporting (CSRD) challenges Western Balkans producers operating without established data networks nor any evidences. This paper proposes the cost-neutral "ESG-Admin-BiH" model, operationalizing the FAO "Proxy Approach" to bridge this gap. Methodologically, the model utilizes a correlation analysis between financial data (AOP codes) and environmental pressures, combined with a deductive scoring system based on audit reports and cross-compliance records. Validated in BiH but regionally scalable, the model synthesizes mandatory financial data (AOP codes) and administrative records into 10 KPIs using a hybrid scoring mechanism. A key innovation is its stratified application: while organic audits verify niche producers, conventional farms are assessed using Cross-Compliance records from the Farm Registry (Registar poljoprivrednih gazdinstava - RPG). Results confirm that existing administrative data can be transformed into robust sustainability assessment systems. We recommend Ministries of Agriculture automate this algorithm within RPG software to facilitate immediate ESG benchmarking for both sectors.

Keywords: Proxy Approach, ESG Reporting, Organic Farms Audits, Cross-Compliance, Sustainable Agriculture

INTRODUCTION

In the era of accelerated transition towards sustainable food systems, defined by the European Green Deal and the "Farm to Fork" strategy, the agricultural sector faces an existential challenge: the imperative to quantify sustainability. While the agricultural sector, particularly livestock production, serves as a fundamental pillar for food security, it is simultaneously recognized as a major source of environmental pressure, including greenhouse gas emissions and biodiversity loss (Lesschen et al., 2011; Tuomisto et al., 2012). Consequently, European Union (EU) markets, which are the primary export destinations for products from Bosnia and Herzegovina (BiH), increasingly demand complex evidence of Environmental, Social, and Governance (ESG) performance.

This pressure is intensified by regulatory mechanisms such as the Corporate Sustainability Reporting Directive (CSRD). Through the "cascade effect," reporting obligations are transferred from large EU buyers down to their suppliers, effectively transforming ESG data collection from a voluntary activity into a mandatory condition for market access (Witt et al., 2025). However, for countries in transition like BiH, this creates a "data paradox". While global supply chains demand transparency "from farm to fork," domestic producers operate in an environment of limited technical capacity and fragmented legislation. Unlike EU member states, BiH has not yet fully established a Farm Accountancy Data Network (FADN) or a Farm Sustainability Data Network (FSDN). Recent regional studies highlight this gap, emphasizing that while the transition to sustainability reporting is inevitable, the lack of structured data remains a critical barrier for Western Balkan farmers (Simin et al., 2025). Furthermore, traditional methodological approaches, such as ISO 14040 Life Cycle Assessments (LCA), which require granular measurement of physical inputs and outputs, have proven prohibitively expensive and inapplicable for the structure of small and medium-sized farms in the region (Wohlenberg et al., 2022).

To bridge this gap, this paper proposes the "ESG-Admin-BiH" model, an innovative conceptual framework that operationalizes the "Proxy Approach" recommended by FAO (Tubiello et al., 2025) for monitoring sustainable development goals in data-scarce environments. The central hypothesis of this research is that compliance data effectively serves as sustainability data. This premise is grounded in the theoretical framework of "green accounting" and Data Envelopment Analysis (DEA), which demonstrate that financial outputs can accurately model ecological pressures when direct physical measurements are unavailable (Picazo-Tadeo, Gómez-Limón, Reig-Martínez, 2011; Sklavos et al., 2025). Financial flows on a farm are not merely monetary transactions but digital footprints of physical material and energy flows; for instance, fuel costs can serve as a direct financial proxy for CO₂ emissions.

The objective of this paper is to present a model that utilizes existing, legally mandatory administrative data streams - specifically financial statements and organic certification audit records aligned with Regulation (EU) 2018/848 — to construct robust sustainability indicators (European Parliament and the Council of the European Union, 2018). This aligns with the broader EU strategy of evolving agricultural data networks from purely economic to multidimensional sustainability tools (Turchetti et al., 2021). While the model is designed to be applicable to all registered agricultural entities, this research focuses on organic farms as the "golden standard" and the initial point of validation, given their rigorous documentation requirements.

MATERIALS AND METHODS

Regulatory Framework and Data Sources

The research analyzes the dual administrative architecture of the agricultural sector in Bosnia and Herzegovina (BiH), encompassing both entities – the Federation of BiH (FBiH) and Republika Srpska (RS). The primary material for the model construction consists of two distinct data streams available for registered agricultural entities:

- **Official financial statements:** The analysis utilizes the standardized Chart of Accounts defined by the Rulebook on the Chart of Accounts for corporate entities in RS and FBiH (Federalno ministarstvo finansija, 2021; Ministarstvo finansija Republike Srpske, 2022). Specifically, the research isolates "Automatic Data Processing" (AOP) codes from the Income Statement that correspond to material costs, energy consumption, and labor costs.
- **Audit records for organic certification:** For qualitative validation, the model integrates control checklists used by accredited certification bodies (e.g., "Organska kontrola") aligned with the ISO 17065 standard and Regulation (EU) 2018/848. These documents provide verified data on "process" indicators such as crop rotation, animal welfare, and risk management.

Methodological Approach: The Proxy Concept

The methodological framework is grounded in the "Proxy Approach" recommended by the FAO for monitoring SDG Indicator 2.4.1 in data-scarce environments (Tubiello et al., 2025). The central premise of the "ESG-Admin-BiH" model is that financial flows serve as a digital footprint of physical material and energy flows. This approach draws upon the theoretical principles of Data Envelopment Analysis (DEA) and "Green Accounting," which demonstrate that financial inputs (e.g., fuel costs) can accurately model ecological pressures (e.g., CO₂ emissions) when direct physical measurements are unavailable (Picazo-Tadeo, Gómez-Limón, Reig-Martínez, 2011; Sklavos et al., 2025). Furthermore, the concept of Economic and Environmental Accounts for Agriculture (EEAA) validates the integration of private costs and social externalities into a single reporting framework (Verhaegen et al., 2004).

The model also adopts the logic of the Life Cycle Sustainability Assessment (LCSA), integrating Environmental (LCA), Economic (LCC), and Social (S-LCA) dimensions (Padilla-Rivera, Hannouf, Assefa, Gates et al., 2024). However, instead of requiring new field measurements, the LCSA framework is adapted for "desk-top" evaluation using existing administrative registries as data points.

Model Construction and Indicator Selection

To operationalize this framework, a comparative analysis of the regulatory requirements was conducted to identify overlapping data points. The selection of Key Performance Indicators (KPIs) was guided by the

principles of availability, relevance, and cost-neutrality, consistent with the 'Proxy Approach' guidelines for data-scarce agricultural systems defined by Tubiello et al. (2025), as well as the constraints of family farming monitoring highlighted in recent literature (Wohlenberg et al., 2022). The adoption of the ESG (Environmental, Social, Governance) framework over the traditional tripartite division of sustainability (economic, social, environmental) was a strategic choice driven by current regulatory and market demands. With the implementation of the Corporate Sustainability Reporting Directive (CSRD), EU supply chains increasingly require sustainability data structured explicitly within the ESG matrix (Witt et al., 2025). Furthermore, the selection of financial ratios as proxy indicators is supported by literature emphasizing the need for cost-effective, farm-level sustainability metrics in data-scarce environments, where standard physical measurements are financially prohibitive for smaller holdings (Latruffe et al., 2016).

RESULTS AND DISCUSSION

The 'ESG-Admin-BiH' Indicator Matrix

The primary result of this research is the definition of the "ESG-Admin-BiH" conceptual model. Unlike traditional Life Cycle Assessments (LCA) which require granular physical data often unavailable to smallholders in the Western Balkans, this model utilizes a "proxy" approach. By mapping the specific Automatic Data Processing (AOP) codes from the official Income Statements and standard checkpoints from ISO 17065 organic audits, we constructed a matrix of 10 Key Performance Indicators (KPIs). Table 1 presents the integrated evaluation matrix, categorized by Environmental (E), Social (S), and Governance (G) dimensions.

Table 1. The 'ESG-Admin-BiH' Indicator Matrix: Data Sources and Scoring Logic

Dimension	ID	Indicator Name	Data Source (BiH Framework)	Proxy Logic (Rationale)
Environmental	E1	Input Intensity	Income Statement (AOP 221 (RS); AOP 256 (FBiH) / AOP 201)	<i>Ratio of material costs to revenue.</i> Lower ratio indicates closed-cycle farming and lower indirect (Scope 3) emissions.
	E2	Energy Efficiency	Income Statement (AOP 222 (RS); AOP 257 (FBiH) / AOP 201)	<i>Ratio of fuel/energy costs to revenue.</i> Acts as a direct financial proxy for carbon intensity and fossil fuel dependence.
	E3	Structural Biodiversity	Audit Report (Section: Environment)	Qualitative score based on the auditor's assessment of landscape features and buffer zones.
	E4	Soil Conservation	Audit Record (Crop Table)	<i>Number of crops in rotation.</i> Verified administrative proof of soil health practices (e.g., 4+ crops = max score).
Social	S1	Labor Contribution	Income Statement (AOP 223 (RS); AOP 258 (FBiH) / AOP 219 (RS); 253 (FBiH))	<i>Share of gross wages in total expenses.</i> Unlike efficiency models, a higher share here validates the social function of rural employment.
	S2	Average Labor Cost	Income Statement (AOP 223 (RS); AOP 258 (FBiH) / No of employees)	<i>Comparison of average labor cost per employee vs. national averages.</i> Proxy for decent work.
	S3	Occupational Safety	Audit Record (Section: Social Justice)	Binary indicator: Existence of any safety non-conformities (NC) zeros the score.
Governance	G1	Regulatory Integrity	Audit Record (Inspection Results)	The "Golden Standard" of governance. Presence of Major Non-Conformities indicates governance failure.
	G2	Economic Viability	Income Statement (AOP 316 (RS); AOP 322 (FBiH) / AOP 201)	<i>Net Profit Margin.</i> Essential for long-term sustainability; a loss-making farm cannot be sustainable.
	G3	Subsidy Independence	Income Statement (AOP 305 (RS); AOP 252 (FBiH) / AOP 201)	<i>Share of subsidies in total revenue.</i> High share (>50%) indicates low market resilience and high political risk.

Interpreting the Environmental Proxies (E) and Scoring Logic

The model rests on the concept of farming eco-efficiency, which posits that financial expenditures on material inputs serve as reliable proxies for environmental pressures (Picazo-Tadeo, Gómez-Limón, Reig-Martínez, 2011). In a market economy, every material input implies a cost, meaning that financial flows can mirror physical flows. Therefore, the 'Input Intensity' indicator (E1) serves as a baseline financial proxy for the ecological footprint. However, it is not an absolute measure of eco-efficiency. A critical limitation of using aggregated cost data is that it is 'quality-blind'. For instance, an organic farm using expensive biopesticides might have the same E1 ratio as a conventional farm using synthetic pesticides, yet their actual ecological efficiencies differ drastically. Similarly, if an organic farm purchases organic manure externally rather than generating it on-farm, its E1 ratio will increase, falsely signaling lower circularity despite maintaining ecological compliance. For organic farms in BiH, which are legally prohibited from using synthetic fertilizers under Regulation (EU) 2018/848, a lower E1 ratio confirms the effective use of on-farm resources (e.g., manure), aligning with circular economy principles. To quantify this, the model employs a scoring methodology adapted from the FAO SAFA Guidelines, which assigns performance ratings based on pre-defined quantitative thresholds (FAO, 2014). Based on the analysis of financial structures in the region, specific thresholds have been defined. For indicator E1, derived from AOP 221 (RS) or AOP 256 (FBiH), a ratio of material costs to total revenue (AOP 201) below 30% is awarded the maximum score (10 points). This threshold reflects the core principle of organic farming: the "closed cycle" system. A farm that spends less than 30% of its revenue on external inputs (fertilizers, seeds) is effectively utilizing on-farm resources (e.g., manure, saved seeds), thereby minimizing Scope 3 emissions associated with the production and transport of industrial inputs (Walczak, Bieda, 2023). Conversely, a ratio exceeding 60% receives zero points, indicating high dependency on external resources and lower ecological resilience.

Similarly, while direct measurement of CO₂ requires expensive sensors, the "Energy Efficiency" indicator (E2) uses fuel costs (AOP 222 in RS and AOP 257 in FBiH) to identify "hotspots" of inefficiency. It must be noted that AOP 222 (RS) aggregates all energy costs without distinguishing between fossil fuels and renewable energy sources. Therefore, while this ratio indicates general energy efficiency, it cannot be strictly interpreted as a metric of carbon neutrality unless supplemented by detailed sub-analytical accounting. Studies on family farming sustainability confirm that the ratio of fuel costs to revenue serves as a precise financial proxy for emission intensity (Wohlenberg et al., 2022). A farm with disproportionately high fuel costs per unit of revenue is invariably a high-emission emitter, regardless of the specific crop. Therefore, the model penalizes inefficiency: ratios above 10% indicate "energy hotspots" and result in a lower score.

For non-financial indicators like 'Soil Conservation' (E4), the model utilizes a quantitative assessment methodology consistent with the institutional shift towards performance-based monitoring described by Turchetti et al. (2021). The auditor's verification of the crop rotation plan is converted into data: a rotation involving 4 or more distinct crops awards 10 points, while a monoculture results in 0 points. This incentivizes polyculture, which is scientifically linked to enhanced soil microbiome resilience and stability (Azarbad, 2022). While crop rotation is utilized here as the primary administrative proxy due to its mandatory recording in audit crop tables, a comprehensive soil conservation assessment could also incorporate secondary verified data points, such as no-tillage cultivation practices, intercropping, and organic manure application, where data is available.

Social and Governance Dimensions: The Deductive Audit Scoring

While environmental data is derived largely from financial ratios, the Social (S) and Governance (G) dimensions rely heavily on the "process data" captured during organic certification audits. Here, the model applies a binary scoring logic consistent with the treatment of categorical governance variables in efficiency analysis described by Sklavos et al. (2025). This reflects the strict nature of compliance standards where non-conformity implies a governance failure.

The "Regulatory Integrity" indicator (G1) is the "Golden Standard" of governance. It assumes a baseline of 10 points, from which points are deducted for non-compliance. The presence of any "Major Non-Conformity" (Major NC) in the inspection report (ISO 17065 audit) automatically triggers a score of zero for this indicator. Minor non-conformities result in proportional deductions (e.g., -1 point per

occurrence). This logic effectively differentiates between farms that are merely "certified" and those that demonstrate proactive, high-quality management.

Furthermore, the "Labor Contribution" indicator (S1) challenges the conventional economic wisdom where labor is solely a cost to be minimized. In the context of sustainable rural development, labor indicators derived from accounting data (such as gross wages and hours worked) are recognized as valid proxies for social contribution and local employment (Coppola et al., 2022). Consequently, labor-intensive organic farming is valued positively within this model as it supports rural vitality.

In the context of sustainable rural development, labor indicators derived from accounting data (such as gross wages) are treated positively as they support local employment and counter rural depopulation. However, it is important to acknowledge that indicators S1 and S2 are primarily applicable to registered agricultural enterprises and cooperatives. For the broader application of this model to family farms — here formal wages are often not paid and the labor force consists mainly of family members — these indicators must be adapted. In such cases, labor inputs should be measured in physical units, specifically the Annual Work Unit (AWU), to accurately reflect the social contribution. Additionally, while increased labor expenditure indicates support for rural vitality, it must be carefully balanced against labor efficiency to avoid penalizing necessary technological advancements.

Composite Scoring and Benchmarking

To facilitate benchmarking between farms, the individual indicator scores are aggregated into a composite ESG Rating (0-100). The weighting system is inherent to the matrix structure: the Environmental dimension, containing 4 indicators, accounts for 40% of the total score, while Social and Governance dimensions account for 30% each.

Based on the total score, farms are classified into four sustainability tiers. This classification system is conceptually adapted from the Farm Sustainability Assessment (FSA) benchmarking framework developed by the SAI Platform (SCS Global Services, 2018), but recalibrated for the specific context of the Western Balkans:

- Platinum (90-100): Leaders in sustainability, characterized by closed-cycle production and zero non-conformities.
- Gold (75-89): Standard high-performing organic farms.
- Silver (50-74): Farms in transition or with structural weaknesses (e.g., high subsidy dependence).
- Bronze (< 50): High-risk operations that may satisfy minimum legal requirements but lack resilience.

Discussion: Organic vs. Conventional Application

The proposed "ESG-Admin-BiH" model aligns with the broader paradigmatic shift in European agricultural statistics, specifically the transition from the Farm Accountancy Data Network (FADN) to the Farm Sustainability Data Network (FSDN). As noted by Turchetti et al. (2021), the integration of environmental and social variables into existing accounting frameworks is a more efficient strategy than creating parallel monitoring systems. By utilizing AOP codes found in standard financial reports, this model demonstrates that BiH can initiate this transition even before full institutional FSDN implementation. Nevertheless, it must be strongly emphasized that relying solely on aggregate accounting data can lead to erroneous sustainability conclusions and ratings due to the inability to isolate price effects from physical practices. FADN/AOP data must be seen as an interim tool, whereas moving towards the holistic FSDN database remains the ultimate objective for the non-organic sector.

Furthermore, the applicability of this model addresses a critical gap in the Western Balkans. Recent regional research by Simin et al. (2025) highlights the scarcity of sustainability studies based on accounting data in the region. Their findings suggest that analyzing the structure of subsidies – distinguishing between investment support and direct payments – is crucial for assessing long-term resilience. Our model incorporates this insight through indicator G3 (Subsidy Independence), treating high dependency on public funds as a governance risk rather than a revenue benefit. This metric evaluates financial resilience, not ecological merit. If two farms—one conventional and one organic—both operate with a 100% subsidy dependency, they both exhibit an identical 'governance risk' due to their extreme vulnerability to shifting agricultural policies and lack of market competitiveness.

Finally, the strong emphasis on the Governance (G) dimension in our matrix is supported by the theoretical work of Sklavos et al. (2025). Their application of Data Envelopment Analysis (DEA) indicates that governance indicators are reliable predictors of "green accounting" quality and eco-efficiency. This validates our decision to use organic audit certifications – essentially a governance mechanism – as a proxy for environmental performance. However, consistent with the recommendations of Verhaegen et al. (2004) regarding Economic and Environmental Accounts (EEAA), future iterations of this model should aim to monetize these externalities more precisely to fully integrate private costs with social benefits.

A recognized limitation of the model is its sensitivity to external price shocks (e.g., fuel inflation). To mitigate this, literature suggests the use of multi-year averages for more accurate economic sustainability benchmarking (Jalić et al., 2022). Furthermore, while the use of financial indicators (such as the ratio of material costs to revenues) offers a pragmatic proxy for environmental pressure, it presents certain methodological limitations that must be acknowledged. First, financial proxies are sensitive to external price volatility; for instance, a sharp increase in fertilizer prices combined with a simultaneous reduction in physical application might paradoxically worsen the economic score while improving the actual environmental footprint. Secondly, standard income statements fail to capture unsold production, such as feed or grain used internally on the farm. Therefore, these proxies are most effective as a rapid 'desk-top' screening tool for commercial entities, whereas the precise isolation of physical volumes from market prices will eventually require the full institutional implementation of the FSDN.

Also, a critical constraint of the proposed model lies in the data asymmetry between organic and conventional producers. Organic farms, by definition, operate in a "data-rich" environment due to mandatory third-party verification required by Regulation (EU) 2018/848 (European Parliament and the Council of the European Union, 2018). Consequently, they possess readily available verified data for "process indicators" such as Structural Biodiversity (E3) and Soil Conservation (E4).

For conventional producers, the application of the 'ESG-Admin-BiH' model requires a stratified approach. The financial proxies (E1, E2, S1, G2, G3) remain fully applicable, as conventional entities are subject to the same financial reporting standards as their organic counterparts (Federalno ministarstvo finansija, 2021; Ministarstvo finansija Republike Srpske, 2022). This allows for immediate comparative analysis of economic efficiency and resource intensity. For instance, comparative LCA studies suggest that conventional systems often exhibit superior scores in "Input Intensity per unit of product" due to higher yields, but inferior scores per unit of area (Tuomisto et al., 2012; de Ponti, Rijk, van Ittersum, 2012).

However, the "Governance" (G) and "Social" (S) dimensions present a challenge due to the absence of the ISO 17065 audit report listed in Table 1. To bridge this gap, the model necessitates a data source substitution. Instead of the audit report, cross-compliance records from the Farm Registry (RPG) are identified as the only valid administrative alternative. Although less granular than organic audits, European studies confirm that administrative checks for Cross-Compliance (Good Agricultural and Environmental Condition – GAEC standards) serve as a sufficient baseline proxy for environmental governance (European Court of Auditors, 2016). Even when physical field checks are limited to a small sample, the administrative universality of these requirements provides a sufficient basis for risk assessment (de Vries et al., 2012). Under this adapted framework, conventional farms would receive a "base score" for complying with minimum legal standards, while organic farms would receive "bonus points" reflecting their voluntary, higher-standard commitments verified by certification.

It must be emphasized, however, that the application of administrative cross-compliance records (RPG) for conventional farms solely substitutes the 'process' and 'governance' indicators. The financial data (AOP codes) required for the environmental proxies remain accessible only for those agricultural entities legally obliged to maintain formal double-entry accounting (commercial enterprises and registered agricultural entrepreneurs). Consequently, non-commercial smallholders operating without standard bookkeeping currently fall outside the scope of the financial proxy evaluation, which further underscores the urgency of deploying a comprehensive FSDN system.

CONCLUSION

This research demonstrates that the current lack of a fully established FADN system in Bosnia and Herzegovina does not preclude the possibility of initial ESG reporting, but rather serves as a stepping stone toward an immediate leap to a full FSDN framework. By operationalizing the FAO "Proxy Approach," the proposed "ESG-Admin-BiH" model successfully transforms mandatory bureaucratic data – specifically Income Statement AOP codes and verified Audit Checklists (or Cross-Compliance records from the Farm Registry) – into valuable sustainability metrics.

A key methodological contribution of this paper is the definition of a hybrid scoring mechanism. By combining scalar scoring for financial-ecological proxies (E1, E2) with deductive scoring for governance compliance (G1), the model generates a composite "ESG Rating" (Platinum, Gold, Silver, Bronze). This granular quantification moves beyond simple "compliant/non-compliant" binaries, allowing for precise benchmarking of farm resilience.

The findings confirm that organic farms, regulated under Regulation (EU) 2018/848, currently represent the "Golden Standard" for data availability. However, the study identifies a viable pathway for conventional producers through a stratified approach. By substituting third-party audit reports with administrative cross-compliance records from the Farm Registry (RPG), the model becomes a universal tool for the wider agricultural sector, albeit with lower process granularity.

For policymakers and administrative bodies, the implication is strategic: existing administrative registries are an underutilized reservoir of sustainability data. We recommend a dual implementation strategy. First, organic certification bodies should introduce an 'ESG Annex' to standard contracts for immediate data capture. Simultaneously, we advise the Entity Ministries of Agriculture (MPŠV RS and FMPVŠ) to integrate this scoring algorithm into the Farm Registry (RPG) software. This would enable the automated transformation of existing Cross-Compliance records into national sustainability ratings, positioning BiH not merely as a passive recipient of EU regulations (CSRD), but as a proactive innovator in digital agriculture. Furthermore, a more advanced policy approach should go beyond mere monitoring. Ministries could utilize this integrated ESG status to actively condition the desired agricultural progress by linking the granting of agricultural subsidies directly to these scores—rewarding strict cross-compliance and the application of environmentally sensitive practices.

REFERENCES

- Azarbad, H. (2022). Conventional vs. Organic Agriculture—Which One Promotes Better Yields and Microbial Resilience in Rapidly Changing Climates? *Frontiers in Microbiology*, 13: 903500. <https://doi.org/10.3389/fmicb.2022.903500>
- Coppola, A., Amato, M., Vistocco, D., & Verneau, F. (2022). Measuring the economic sustainability of Italian farms using FADN data. *Agricultural Economics – Czech*, 68, 327–336. <https://doi.org/10.17221/169/2022-AGRICECON>
- de Ponti, T., Rijk, B., & van Ittersum, M. K. (2012). The crop yield gap between organic and conventional agriculture. *Agricultural Systems*, 108, 1–9. <https://doi.org/10.1016/j.agsy.2011.12.004>
- de Vries, W., Kros, J., Voogd, J. C., & Reidsma, P. (2012). *Impacts of cross compliance measures on environmental indicators: an assessment tool to evaluate environmental impacts at the European scale*. Wageningen: Alterra.
- European Court of Auditors. (2016). *Making cross-compliance more effective and achieving simplification remains challenging* (Special Report No 26/2016). Luxembourg: Publications Office of the European Union.
- European Parliament and the Council of the European Union. (2018). Regulation (EU) 2018/848 on organic production and labelling of organic products. *Official Journal of the European Union*, L 150, 1–92.
- FAO. (2014). *SAFA Guidelines – Sustainability Assessment of Food and Agriculture systems (Version 3.0)*. Rome: FAO.

- Federalno ministarstvo finansija. (2021). Pravilnik o sadržaju i formi finansijskih izvještaja za privredna društva. *Službene novine Federacije BiH*, 81/21.
- Jalić, M., Rozman, Č., Vaško, Ž., & Pažek, K. (2022). Determining the Extent of Economical Sustainability of a Case Study Milk Farm in Bosnia and Herzegovina Based on the Real Options Model. *Sustainability*, 14(19), 11993. <https://doi.org/10.3390/su141911993>
- Latruffe, L., Diazabakana, A., Bockstaller, C., Desjeux, Y., Finn, J., Kelly, E., Ryan, M., & Uthes, S. (2016). Measurement of sustainability in agriculture: a review of indicators. *Studies in Agricultural Economics*, 118(3), 123-130. <http://dx.doi.org/10.7896/j.1624>
- Lesschen, J. P., van den Berg, M., Westhoek, H. J., Witzke, H. P., & Oenema, O. (2011). Greenhouse gas emission profiles of European livestock sectors. *Animal Feed Science and Technology*, 166-167, 16-28. <https://doi.org/10.1016/j.anifeedsci.2011.04.058>
- Ministarstvo finansija Republike Srpske. (2022). Pravilnik o sadržini i formi obrazaca finansijskih izvještaja za privredna društva, druga pravna lica i preduzetnike. *Službeni glasnik Republike Srpske*, 59/22.
- Picazo-Tadeo, A. J., Gómez-Limón, J. A., & Reig-Martínez, E. (2011). Assessing farming eco-efficiency: a Data Envelopment Analysis approach. *Journal of Environmental Management*, 92(4), 1154-1164. <https://doi.org/10.1016/j.jenvman.2010.11.025>
- SCS Global Services. (2018). *Farm Sustainability Assessment 2.1 – User Guide*. SAI Platform. Retrieved from https://cdn.scsglobalservices.com/files/program_documents/fsa_onfarm_userguide_may2018.pdf
- Simin, M. T., Glavaš-Trbić, D., Miljatić, A., Despotović, A., & Novaković, T. (2025). Farm Sustainability Indicators—Exploring FADN Database. *Land*, 14(10), 1950. <https://doi.org/10.3390/land14101950>
- Sklavos, G., Zournatzidou, E., Ragazou, K., & Sariannidis, N. (2025). Green accounting and ESG-driven eco-efficiency in European financial institutions: A two-stage DEA–CRITIC–TOPSIS evaluation. *PLoS One* 20(10): e0334882. <https://doi.org/10.1371/journal.pone.0334882>
- Tubiello, F.N., Wanner, N., Kalamvrezos Navarro, D., Bizier, V., Khan, A.A. & Bacci, S. (2025). Proxy approach for status and trend assessments of productive and sustainable agriculture. *FAO Statistics Working Paper Series, No. 25-50*. Rome, FAO. <https://doi.org/10.4060/cd7417en>
- Tuomisto, H. L., Hodge, I. D., Riordan, P., & Macdonald, D. W. (2012). Does organic farming reduce environmental impacts? – A meta-analysis of European research. *Journal of Environmental Management*, 112, 309-320. <https://doi.org/10.1016/j.jenvman.2012.08.018>
- Turchetti, B., Gastaldin, D., & Marongiu, S. (2021). Enhancing the Italian FADN for sustainability assessment: The state of art and perspectives. *Economia agro-alimentare / Food Economy*, 23(3). <https://doi.org/10.3280/ecag2021oa12771>
- Verhaegen, I., Wustenberghs, H., Lauwers, L., & Mathijs, E. (2004). *Integrated Economic and Environmental Accounting for Agriculture*. Centre for Sustainable Agriculture.
- Witt, N., Thorsøe, M.H., & Graversgaard, M. (2025). ESG reporting meets farmer – implications of the European corporate sustainability reporting directive for the agrifood sector. *British Food Journal*, 127(13), 264–283. <https://doi.org/10.1108/BFJ-01-2024-0110>
- Wohlenberg, J., Schneider, R. C. S., & Hoeltz, M. (2022). Sustainability indicators in the context of family farming: A systematic and bibliometric approach. *Environmental Engineering Research*, 27(1), 200545. <https://doi.org/10.4491/eer.2020.545>

Corporate valuation of agricultural enterprises for informed investment decision-making

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Abstract

The study evaluated the financial performance and valuation indicators of selected agricultural corporations listed on the Croatian capital market during the period 2021–2025. The analysis includes Kutjevo p.l.c., Koka Varaždin p.l.c., Croatian Sugar Industry p.l.c., Žito Group p.l.c., and Podravka p.l.c. The research applied a quantitative approach based on secondary financial data obtained from annual reports, the Zagreb Stock Exchange, and the Financial Agency (FINA). The valuation framework is based on three standard financial models: discounted cash flow (DCF), Capital Asset Pricing Model (CAPM), and Economic Value Added (EVA). Comparative and descriptive analyses were used to examine differences in asset values, expected returns on equity, and value creation among companies. The results indicate significant differences in financial scale and performance indicators across the analyzed corporations. Larger companies, particularly Podravka p.l.c. and Žito Group p.l.c., recorded higher total asset values and EVA results, while smaller companies demonstrated lower but relatively stable financial performance. CAPM-based estimates of expected returns show moderate variation across firms, reflecting differences in systematic risk and market positioning. The findings suggest that valuation indicators provide useful comparative information for assessing financial performance in capital-intensive agricultural sectors. However, the results should be interpreted as descriptive evidence rather than causal relationships due to methodological limitations related to secondary data analysis and model assumptions.

Keywords: agricultural companies, company valuation, CAPM, EVA, financial performance

INTRODUCTION

Corporate valuation represents an important component of financial analysis and strategic decision-making in modern business systems. The primary purpose of valuation is to estimate the economic value of a company based on available financial and market information, thereby providing support for investment decisions, corporate governance, and long-term business planning (Pavlović, 2008). Company valuation represents a comprehensive analytical process that integrates all relevant information about an enterprise, including financial statements, value drivers, statistical forecasts, determinants of value, and performance indicators, through the application of valuation methods aimed at providing an expert opinion on the value of a company at a specific point in time (Necula, 2023). In corporate finance theory, company value is commonly understood as the present value of expected future cash flows, adjusted for the level of investment risk and the required rate of return (Helfert, 2000). Accordingly, valuation procedures require a detailed assessment of financial statements, profitability

indicators, capital structure, liquidity, investment activities, and overall market performance. In recent years, environmental, social, and governance (ESG) criteria have become increasingly important for investors and financial institutions in making financing and investment decisions (Mentes, 2023). Through this perspective, external costs, particularly the negative impacts that corporate activities impose on society, are increasingly internalized within economic evaluation frameworks. This contributes to the development of a system in which sustainable business practices become economically advantageous, while unsustainable corporate behavior is systematically discouraged (Zubović, 2024).

Financial analysis aims to provide insight into the financial position and operational performance of a company through the interpretation of accounting and market-based indicators (Raković, 2010). In this context, valuation methods are frequently used to compare companies operating within the same sector and to identify differences in profitability, efficiency, and risk exposure. Company performance is influenced by numerous internal and external factors, including managerial decisions, capital investments, market competition, input costs, and broader economic conditions. Therefore, objective financial analysis requires the integration of several complementary indicators in order to provide a realistic assessment of corporate performance and investment potential (Pavlović, 2008).

Economic theory distinguishes several approaches to company valuation (Kronholm, 2013). Economic value assessment focuses on the company's ability to generate sustainable future cash flows and shareholder returns, while market value reflects the price determined through interactions between buyers and sellers in the capital market. Book value is derived from accounting data presented in financial statements and represents the difference between total assets and liabilities. Book value is primarily used in the valuation of companies that are not expected to continue as going concerns (Pinto et al., 2020). Consequently, the book values of assets reported in the balance sheet often bear limited relevance to their actual economic value. The balance sheet therefore serves mainly as a starting point for valuation and requires a series of adjustments in order to arrive at fair market value (Hitchner, 2017). The valuation process involves the application of quantitative financial methods designed to estimate the relationship between expected returns and investment risk. Among these, the discounted cash flow (DCF) method is one of the most widely applied valuation approaches, as it estimates the value of an investment based on its expected future cash flows. Specifically, DCF analysis enables analysts to determine the present value of an investment by discounting projected future cash flows to reflect both the time value of money and the associated level of risk, thereby providing an estimate of the investment's current worth (Fernando, 2024).

Investment activities represent a key determinant of corporate growth and competitiveness. Investments are associated with uncertainty and risk because their outcomes depend on future market conditions, operational efficiency, and strategic decision-making (Hirt et al., 2005). Longer investment horizons generally involve greater uncertainty, emphasizing the importance of reliable financial analysis and risk assessment in investment planning (Pike, 2009). Inadequate investment decisions or insufficient information may negatively affect business performance and value creation (Litterman, 2003). Therefore, investment analysis should be based on systematic financial evaluation rather than ad hoc decision-making approaches (Šišek, 2005). From an investment perspective, companies that operate sustainably are perceived as less risky and more stable in the market (Biancardo et al., 2023). The agricultural sector represents a strategically important segment of the Croatian economy due to its role in food production, rural development, and participation in domestic and international value chains (Arsenović et al., 2002). Agricultural corporations are characterized by significant capital intensity, requiring substantial investments in land, machinery, production facilities, logistics infrastructure, and processing capacities. As such, they are often oriented toward less intensive forms of agricultural production, particularly mixed farming systems and livestock grazing (Očić et al., 2022). In recent years, agribusiness companies have increasingly adopted vertically integrated business models that combine primary production, processing, and distribution activities in order to enhance operational efficiency and market competitiveness. Such organizational structures may contribute to differences in financial performance and company valuation among agricultural enterprises. This form of business organization is typically associated with combined production processes that generate multiple outputs depending on the physical and chemical characteristics of production, thereby complicating their reliable valuation. The complexity of valuation is further intensified by the bidirectional interdependence between crop and livestock production in the estimation of recoverable costs (Raboshuk et al., 2022). Given the importance of financial sustainability and

investment efficiency in the agricultural sector, the application of valuation methods can provide valuable insights into the comparative financial position and performance of agricultural corporations operating in capital markets. In this context, it is essential to examine operational performance indicators in relation to resource utilization activities, as these provide critical information for measuring efficiency, profitability, and liquidity (Boiko et al., 2024).

Accordingly, the main objective of this study was to evaluate the financial performance and valuation indicators of selected agricultural corporations operating on the Croatian capital market during the period 2021–2025. The study applies established valuation approaches, including discounted cash flow (DCF), Capital Asset Pricing Model (CAPM), and Economic Value Added (EVA), in order to compare company performance, asset structure, and value creation potential.

MATERIAL AND METHODS

The research was conducted on selected agricultural and agri-food companies operating on the Croatian capital market during the period 2021–2025. The analysis included corporations listed on the Zagreb Stock Exchange and companies with publicly available financial statements and market data. The selected companies were Kutjevo p.l.c., Koka Varaždin p.l.c., Croatian Sugar Industry p.l.c., Žito Group p.l.c., and Podravka p.l.c.

The study employed a quantitative research approach based on secondary financial data obtained from publicly available sources, including annual financial reports, the Zagreb Stock Exchange, and the Financial Agency (FINA). These sources were selected because of their official status, methodological reliability, and accessibility. The collected data included information on total assets, share prices, cash flows, investments, and profitability indicators relevant for company valuation analysis.

The analytical framework combined descriptive, comparative, and financial analysis methods in order to evaluate differences in company size, financial performance, and value creation among the analyzed corporations. The following methodological procedures were applied:

- Descriptive analysis – presentation of financial and valuation indicators of agricultural corporations;
- Comparative analysis – comparison of company valuation indicators during the observed period;
- Financial analysis – assessment of expected returns, asset values, and value creation using selected valuation models;
- Statistical analysis – calculation of basic indicators of variability and comparison among companies.

The valuation analysis was based on three commonly applied financial approaches: discounted cash flow (DCF), Capital Asset Pricing Model (CAPM), and Economic Value Added (EVA).

The discounted cash flow approach estimates the present value of a company based on expected future cash flows discounted by the required rate of return (Cvetković-Bogavac, 2010):

$$V = \sum_{t=1}^n * \frac{CF_t}{(1 + k_t)^t}$$

Where:

- V – present value of the company,
- CF_t – expected cash flow in period t ,
- k_t – discount rate (required rate of return),
- n – number of observed.

The Capital Asset Pricing Model (CAPM) was applied to estimate the expected return on equity using market risk indicators (Jakovčević, 2000):

$$R_i = R_{rf} + \beta(R_m - R_{rf})$$

Where:

- R_i – expected return on equity,
- R_{rf} – risk-free rate of return,
- β – beta coefficient representing systematic market risk,
- R_m – expected market return.

For the purposes of this study, the risk-free rate was approximated using long-term Croatian government bond yields, while market return estimates were based on average CROBEX market index performance during the observed period. Beta coefficients were estimated using historical market data and comparable sector information available from financial reports and market sources.

Economic Value Added (EVA) was used to assess value creation generated by invested capital (Kačanski et al., 2014):

$$EVA = I(r-WACC)$$

Where:

- *EVA* – economic value added,
- *I* – invested capital,
- *r* – return on invested capital,
- *WACC* – weighted average cost of capital.

Positive EVA values indicate that a company generated returns above the cost of capital, whereas negative values suggest insufficient returns relative to financing costs.

Return on Invested Capital (ROIC) was calculated accordingly to following equation

$$ROIC = \left(\frac{Cash\ flow}{Investment} \right) \times 100$$

The analysis focused on comparing company asset values, expected returns on equity, and EVA indicators during the period 2021–2025. Financial indicators were presented in tabular and graphical form to facilitate comparative interpretation of company performance. All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC, USA), version 9.4.

RESULTS AND DISCUSSION

The empirical analysis of selected agricultural corporations in the Republic of Croatia was conducted using three valuation approaches: Total asset assessment, Capital Asset Pricing Model (CAPM), and Economic Value Added (EVA). The results are presented for the period 2021–2025 and are interpreted in a comparative framework. Table 1 presents the total asset values of the analyzed companies over the observed period.

Table 1. Total company asset values (EUR million), 2021–2025

Company	2021	2022	2023	2024	2025
Kutjevo p.l.c.	35.8	34.4	38.0	39.4	44.4
Koka Varaždin p.l.c.	66.5	69.8	68.5	69.7	71.6
Croatian Sugar Industry p.l.c.	59.2	58.7	60.8	60.3	62.9
Žito Group p.l.c.	440.0	442.3	440.9	444.4	457.1
Podravka p.l.c.	645.4	643.4	647.4	650.3	674.0

Podravka p.l.c. recorded the highest total asset values throughout the observed period, followed by Žito Group p.l.c., while the remaining companies operated at significantly lower asset levels. The observed differences reflect variations in company size, capital structure, and scope of business activities. Figure 1 illustrates the average total asset values of the analyzed companies.

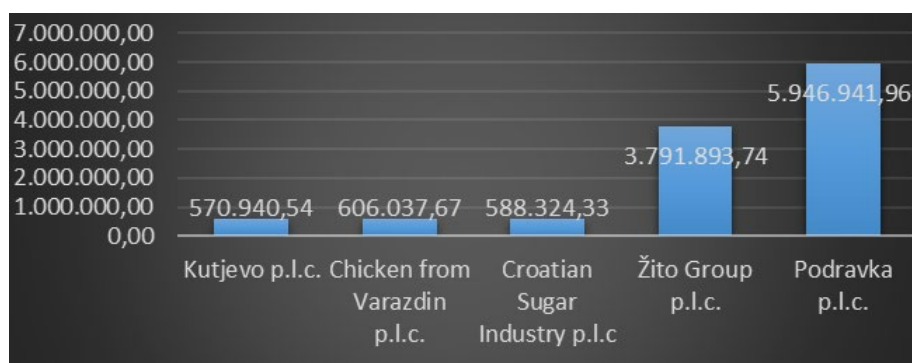


Figure 1. Total asset values of selected agricultural companies (2021–2025), EUR million
Source: Author's calculation based on Zagreb Stock Exchange, FINA, and company annual reports

Podravka p.l.c. clearly dominates in terms of total asset base, with an estimated value exceeding EUR 5,960 million, followed by Žito Group at approximately EUR 3,950 million. The remaining companies operate at a considerably smaller scale. These differences can be associated with differences in operational structure and capital intensity. Larger companies such as Podravka and Žito Group operate through vertically integrated systems that include primary production, processing, and distribution, which typically require higher levels of fixed and working capital. Smaller companies are more specialized and operate within narrower segments of agricultural production. Table 2 presents key financial indicators used for valuation, including expected returns derived from the CAPM model.

Table 2. Key valuation parameters (average 2021–2025)

Company	Share price (EUR)	Cash flow (EUR)	Investments (EUR)	Expected return on equity (%)
Kutjevo p.l.c.	8	3,990,000	16,500,000	9.0
Koka Varaždin p.l.c.	9	4,850,000	20,200,000	10.0
Croatian Sugar Industry p.l.c.	12	4,710,000	19,700,000	8.5
Žito Group p.l.c.	18	35,600,000	120,000,000	10.0
Podravka p.l.c.	140	63,555,000	330,000,000	10.5

Expected returns on equity estimated using CAPM methodology are presented at the Figure 2.



Figure 2. Expected return on equity estimated using the Capital Asset Pricing Model (CAPM) (%)
Source: Author's calculation based on Zagreb Stock Exchange, FINA, and company financial reports

Podravka p.l.c. achieved the highest expected return on equity, followed closely by Žito Group and Koka Varaždin. Kutjevo and the Croatian Sugar Industry recorded slightly lower expected returns. These

results suggest moderate differences in perceived investment risk and return expectations among companies. However, CAPM-based estimates are sensitive to assumptions regarding beta coefficients and market conditions, and therefore should be interpreted as relative rather than absolute performance indicators. Furthermore, the EVA results for the analyzed companies is presented at Figure 3.



Figure 3. Economic Value Added (EVA) of selected agricultural companies (EUR)
Source: Author's calculation based on Zagreb Stock Exchange, FINA, and company financial reports

Podravka p.l.c. generated the highest EVA values, exceeding EUR 250 million, followed by Žito Group with approximately EUR 100 million. The remaining companies recorded significantly lower EVA levels. Positive EVA values indicate that companies generated returns above their cost of capital, suggesting effective capital utilization. However, differences in EVA levels are strongly influenced by company size and invested capital base, meaning that larger companies tend to generate higher absolute EVA values. For this reason, EVA results should be interpreted alongside relative performance indicators rather than in isolation.

The obtained results indicate systematic differences in financial indicators among the analyzed agricultural corporations operating on the Croatian capital market. In particular, larger companies such as Podravka p.l.c. and Žito Group p.l.c. consistently exhibit higher total asset values, expected returns on equity, and Economic Value Added (EVA) compared to smaller and more specialized companies. These findings are consistent across all applied valuation approaches, including DCF, CAPM, and EVA. The observed differences in total asset values (Figure 1) reflect variations in capital structure, operational scale, and business complexity. Companies with broader operational integration and diversified production systems tend to maintain higher asset bases, which is consistent with general principles of corporate finance and investment analysis (Cvetković-Bogavac, 2010). However, these differences should be interpreted primarily as structural characteristics rather than direct indicators of superior efficiency.

The CAPM-based expected returns (Figure 2) suggest relatively moderate variation in risk-adjusted performance among the analyzed companies. Podravka p.l.c. and Žito Group p.l.c. show slightly higher expected returns compared to smaller firms, while Kutjevo p.l.c. and Croatian Sugar Industry p.l.c. exhibit lower but stable values. These results are in line with the theoretical framework of CAPM, where expected return is determined by systematic risk exposure rather than company size alone (Jones 2008). It should be emphasized that CAPM estimates are sensitive to input parameters such as beta coefficients and market return assumptions, and therefore represent model-based approximations rather than observed realized returns.

The EVA results (Figure 3) further highlight differences in value creation among companies. Podravka p.l.c. records the highest EVA values, followed by Žito Group p.l.c., while smaller companies achieve significantly lower absolute EVA levels. According to Kačanski et al. (2014), EVA is a useful indicator of value creation as it measures the surplus generated over the cost of capital. However, in this study, differences in EVA are strongly influenced by differences in invested capital size, which limits direct comparability of absolute values across firms with different scales of operation.

Table 3. Key valuation parameters (average 2021–2025)

Company	Beta coefficient (%)	Risk-free rate (%)	Actual weighted average cost of capital (WACC) (%)	Return on invested capital (ROIC) (%)
Kutjevo d.d.	0,11	2,66	8,05	24,18
Koka Varaždin d.d.	0,14	2,92	8,23	24,01
Croatian Sugar Industry p.l.c	0,19	2,99	7,35	23,91
Žito Grupa d.d.	0,22	3,14	8,61	29,67
Podravka d.d.	0,46	3,48	8,81	19,26

The beta coefficient values, ranging from 0.11 to 0.46, indicate that the companies exhibit a high degree of stability and resilience to market shocks, suggesting that their stocks are likely to remain relatively resistant during periods of market volatility. All observed companies achieve exceptionally high rates of return on invested capital (ROIC), implying that for every EUR 100 of invested capital, they generate between EUR 19 and EUR 30 in profit.

The actual weighted average cost of capital (WACC) values demonstrate that these companies generate returns significantly exceeding their cost of capital, thereby successfully creating value for shareholders and exhibiting strong investment potential. Furthermore, the risk-free rate values, ranging from 2.6% to 3.5%, suggest that the companies were analyzed under conditions of moderate and rising interest rates, largely as a consequence of inflationary pressures. A higher risk-free rate, such as that observed in the market, is likely to increase corporate financing costs and, consequently, contribute to a higher WACC. When all three valuation approaches are considered jointly, a consistent pattern emerges: larger and more diversified companies tend to exhibit higher absolute financial indicators, while smaller firms demonstrate more limited but stable performance levels. This pattern is consistent with general findings in corporate valuation literature, which emphasize the importance of capital base, investment structure, and operational scale in determining financial outcomes (Cvetković-Bogavac, 2010).

Nevertheless, the study does not establish causal relationships between diversification, company size, and financial performance. The results should therefore be interpreted as comparative descriptive evidence rather than proof of structural efficiency advantages. The combination of DCF, CAPM, and EVA methods provides a multidimensional view of company performance, allowing for a more comprehensive assessment of financial position and value creation potential within the agricultural sector.

CONCLUSION

The aim of this study was to evaluate the financial performance and valuation indicators of selected agricultural corporations operating on the Croatian capital market during the period 2021–2025, using discounted cash flow (DCF), Capital Asset Pricing Model (CAPM), and Economic Value Added (EVA) approaches. The results indicate clear differences in financial indicators among the analyzed companies. Podravka p.l.c. and Žito Group p.l.c. consistently recorded higher total asset values, expected returns, and EVA results compared to smaller and more specialized agricultural firms. These differences primarily reflect variations in company size, capital structure, and scope of operations. The CAPM-based analysis suggests relatively moderate variation in risk-adjusted expected returns among companies, while EVA results highlight differences in value creation driven largely by differences in invested capital. Smaller companies demonstrated lower absolute financial indicators but maintained stable performance within their operational scale. The findings should be interpreted as comparative descriptive evidence rather than causal relationships between company size, diversification, and financial performance. Due to the methodological limitations of secondary data analysis and the assumptions embedded in valuation models, the results provide an indicative rather than definitive assessment of corporate performance. Overall, the study demonstrates that the combined application of DCF, CAPM, and EVA methods can provide a useful framework for comparative financial analysis of agricultural corporations. The values indicate that these

are financially sound and profitable companies operating with a low level of risk. All observed firms manage their resources efficiently, as reflected in their high ROIC, demonstrate resilience to market fluctuations through low beta coefficient values, and generate returns that substantially exceed the cost at which capital has been obtained, as evidenced by their WACC. Such an approach may support more informed investment evaluation and contribute to a better understanding of financial differences within capital-intensive sectors such as agribusiness. Future research should consider expanding the dataset, incorporating longer time series, and applying additional econometric methods to improve the robustness and explanatory power of valuation-based analyses.

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REFERENCES

- Arsenović, Đ., Krstić, B. (2002). Konkurentnost poljoprivrednih gazdinstava. Beograd: Poljoprivredni fakultet Univerziteta u Beogradu i Poljoprivredni fakultet Univerziteta u Istočnom Sarajevu, pp. 111-113.
- Biancardo, S.A., Gesualdi, M., Savastano, D., Intignano, M., Henke, I., & Pagliara, F. (2023). An innovative framework for integrating cost-benefit analysis (CBA) within building information modeling (BIM). *Socio-Economic Planning Sciences*, 85, 101495. <https://doi.org/10.1016/j.seps.2022.101495>
- Boiko, V., Lyzak, M., Vasylytsiv, T., Lupak, R., & Ohinok, S. (2024). Financial and economic performance of agricultural enterprises: analysis and policy improvement. *Agricultural and Resource Economics*, 10(4), 129-155. <https://doi.org/10.51599/are.2024.10.04.06>
- Cvetković-Bogavac, N. (2010). Finansijske strategije korporacija. Megatrend Univerzitet, Beograd, pp. 101-109.
- Fernando J. (2024). Discounted cash flow (DCF), pp. 6. Available at: <https://www.investopedia.com/terms/d/DCF.asp>
- Financial Agency (FINA). Available at: <https://www.fina.hr/javne-usluge-za-poslovne-subjekte/registri/registar-godisnjih-financijskih-izvjestaja>
- Hirt, G., & Block, S. (2005). Managing Investments. McGraw-Hill, New York, pp. 91.
- Hitchner, J.R. (2017). Financial valuation: Applications and models. Hoboken, NJ: John Wiley & Sons, pp. 344
- Jakovčević, K. (2000). Struktura i vrednovanje kapitala. Ekonomski fakultet, Subotica, pp. 204-208.
- Jones, C.P. (2008). The Intelligent Portfolio. Practical Wisdom on Personal Investing from Financial Engines. Hoboken: John Wiley & Sons, pp. 239.
- Kronholm, M. (2013). Valuation of unlisted direct investment equity and the impact on Finnish international investment position. Master thesis, Aalto University, School of Business, pp. 194.
- Litterman, B. (2003). Modern Investment Management. John Wiley & Sons, Hoboken, New York, pp. 61.
- Mentes, M. (2023). Sustainable development economy and the development of green economy in the European Union. *Energy, Sustainability & Society*, 13, 32, 113-117.
- Necula, S.C. (2023). Balancing profitability and sustainability: the challenges and opportunities for European businesses in environmental and social policies. Financial and Monetary Policies for Fostering European Integration, p. 162.
- Očić, V., Jež Rogelj, M., Mikuš, O., Batelja Lodeta, K., & Šakić Bobić, B. (2022). Economic and financial analysis of the fruit and olive growing sector in Croatian agriculture. *Agroeconomia Croatica*, 12(2), 90-97.

- Pavlović, V. (2008). Metodologija vrednovanja kapitala i usvojeni model privatizacije u Republici Srbiji.
- Pike, R. (2009). Corporate Finance and Investment. Pearson Education, Harlow, pp. 148.
- Pinto, J.E., Henry, E., Robinson, T.R., & Stowe, J.D. (2010). Equity asset valuation. Hoboken, NJ: John Wiley & Sons, pp 296-299.
- Raboshuk, A., Lehenchuk, S., Valinkevych, N., Polishchuk, I., & Khodakyvskyy, V. (2022). Analysis of financial performance determinants: evidence from Slovak agricultural companies. *Agricultural and Resource Economics*, 8(4), 66-85. <https://doi.org/10.51599/are.2022.08.04.03>
- Raković, T. (2010). Pristupi i metode procjene, diskontna stopa i modeli za utvrđivanje cijene kapitala. Available at: https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fef.unibl.org%2Fliteratura%2F170183IV_Metode_procjene_i_diskontna_stopa.ppt.pptx&wdOrigin=BROWSELINK
- Tadjeddine, Y. (2004). L'évaluation des firmes comme convention. Document de travail, Working Zagreb Stock Exchange. Available at: <https://zse.hr/>
- Zubović, A. (2024). Korporativno izvještavanje o održivosti - Izazovi i prilike za male i srednje poduzetnike. *Zbornik radova Pravnog fakulteta u Splitu*, 61(4), 557-587. <https://doi.org/10.31141/zrpf.2024.61.154.557>