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Editorial

Novel developments in agricultural sciences in the light of agricultural symposia

In the light of the newest developments in the world of agricultural production it is necessary once more to emphasize the importance of science and scientific research. This conference and papers submitted and presented here are one of the most important components in this respect in our region. The research is on topics from all fields of agricultural science and it is important to have from time to time in one place. As many conferences nowadays are narrow and specific for the field and rightfully so, there is a need to have a general conference where the wider aspects of agricultural science and research are discussed in multidisciplinary environments.

The principal aim of this annual conference is to get the scientists from the agricultural field from the region but also from the whole world. This is to present their most recent scientific results and discuss about the future directions in which the agricultural science and production will go. Farmers and scientists are faced with dynamics in the field and disturbances like never before in modern era. Pandemics that occurred in last few years have disturbed the markets, labour distribution and work organization as whole.

Inputs in agriculture have skyrocketed as recent as in the last year. It went down and in the same sudden manner as the global prices declined to the base. Farmers and industries would be lost in those conditions without scientific inputs and academia guiding the way and providing directions to the future trends. The current floods in the region have highlighted the weak points and once again reminded all of us that climate disturbances are real and more frequent than ever in modern times.

Those who fail to adjust and fail to listen, understand and ultimately rely on the scientific research and its findings will continue to struggle at the cost of food shortages and high prices for the basics. This became even more obvious given the recent political disturbances in Europe which let to shortages in grain and sunflower supplies, which reflected in prices of those commodities.

Sustainable use of the resources and its conservation for future generations is on of the major issues this generation of agricultural scientists should have always in mind while doing its work. We can make impressive results now, but how sustainable those results are over the time, and how long we will be able to keep the tempo is the real question. Responsible science and innovation must and basically is obliged to go hand in hand.

This conference proceedings have presents many of the current issues researched. And all of them definitively in the light of in the shade of those events. It is in these Proceedings and in person at the conference that us, agricultural scientists together, present and share the research, issues, problems, findings, results, conclusions and the steps forward. The Proceedings contains 31 papers presented at XII International Symposium on Agricultural Sciences "AgroReS 2023" in Trebinje, Bosnia and Herzegovina, from 24 to 26 May, 2023.

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Effects of cultivar and plant origin on the aeroponic production of potato minitubers

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Abstract

Aeroponics is eco-friendly, soilless technology for the cultivation of vegetable plants that can be used for the production of pathogen-free pre-basic seed potato, namely minitubers. In aeroponic modules, the underground parts of potato plants grow suspended in the mist of finely dispersed nutrient solution to produce tubers, while the shoots grow above the module under greenhouse conditions. This study aimed to evaluate the effects of the cultivar and origin of planting material on the minituber production in an aeroponic facility in Guča, Serbia. Two potato cultivars, Sinora and Agria, and two types of planting material, acclimated microplants and plants originating from sprouted minitubers, were used in the study. Plants were grown in the 2019 season with a planting density of 24 plants per m^2 and ~14-day harvest intervals. Agria plants of both plant origins steadily tuberized during most of the cultivation period and formed a significantly larger number of minitubers (13.61) compared to cultivar Sinora (3.35), which quickly completed the growth cycle. In both investigated cultivars, the mass of formed minitubers was significantly higher in the plants originating from minitubers (18.80 g) compared to plants of *in vitro* origin (9.04 g). Agria plants of minituber origin produced the heaviest minitubers (19.05 g), while Sinora plants of *in vitro* origin formed the least heavy tubers (5.29 g). The highest yield of minitubers, 6.26 kg m^{-2}, was recorded for Agria plants of minituber origin. The results of our study suggest that both plant origin and potato genotype significantly affect minituber production in aeroponics.

Key words: potato, aeroponics, pre-basic seed potato, minitubers

Introduction

The high yields of potato, *Solanum tuberosum* L., largely depend on the use of healthy planting material, that is, pathogen-free seed potato. Conventional seed potato-production currently includes several phases: *in vitro* propagation of pathogen-free plant material (microplants or microtubers), production of minitubers by the cultivation of *in vitro*-obtained plant material on a substrate under controlled environments (greenhouses) and minitubers' vegetative propagation in the field to increase the volume of seed material (Broćić et al., 2021). Minitubers are accepted worldwide as a starting material for the field propagation of seed tubers in potato seed production systems. They are more robust and produce a larger number of stems and, therefore, a more significant number of new tubers per plant than *in vitro*-obtained plantlets and microtubers (Broćić et al., 2021). Minitubers of size over 1 cm are usually used as planting material in field production (da Silva Filho et al., 2020). The low reproduction rate and variable size of minitubers are the main limitations of conventional production (Buckseth et al., 2016). The number of minitubers produced in a substrate usually is 2–5 tubers per cultivated microplant (Struik, 2007). Due to the low minitubers' yield, a common practice in seed production programs is the production of the final seed tubers after three to five generations of cultivation in the open field, with every cycle increasing the risk of pathogen infection (Thomas-Sharma et al., 2016). During the last 20 years, aeroponic systems were established to improve seed potato production (Broćić et al., 2021).

Aeroponics is a modern soilless growing technology that enables the production of a large number of high-quality potato minitubers and reduces the number of multiplication cycles in the field for two to three generations. Besides, plants grown in an aeroponic system are well-protected from pests and soil-borne diseases. In aeroponics, the plant's foliage is grown exposed to greenhouse conditions (insect- and pest-free greenhouses/net houses), while the roots and underground stems grow inside an aeroponic module, suspended in the mist of finely dispersed nutrient solution (Lakkireddy et al., 2012). Consequently, minitubers are formed on underground stems - stolons. Minitubers are harvested upon reaching the desired size, which stimulates the initiation of novel tubers and the enlargement of smaller, uncropped tubers for future collecting (Broćić et al., 2021).

Aeroponics excels in producing seed potatoes due to exceptional yields resulting from plants' vigorous growth, prolonged growing season, and multiple harvests. Previous research revealed that the number of minitubers per plant produced during the season, depending on the cultivar, can range from 36 to over 100 (da Silva Filho et al., 2018; Broćić et al., 2022). Broćić et al.

(2019a) reported that acclimated microplants yielded 5.39 times more minitubers when grown in the aeroponic system (9 harvests) compared to conventional cultivation in substrate. Besides, successive harvesting during the production cycle allows minitubers to reach the desired size. Farran and Mingo-Castel (2006) collected tubers with an average weight of 8 g, considering these tubers optimal in terms of weight and diameter. According to Ritter et al. (2001), the average weight of the minitubers was 13.3 g. Duration of the pre-harvest period (harvest intervals) is also important, and various harvest intervals were reported in the literature, ranging from 7 to 20 days (Broćić et al., 2021). Mbiyu et al. (2012) recommended that the collection of minitubers should be done every 10-14 days.

The starting plant material for the aeroponic production of potato minitubers may include acclimated microplants and microtubers delivered from *in vitro* culture, as well as rooted stem cuttings and rooted shoots obtained from *in vitro*-originated plants after acclimatization (Mbiyu et al., 2018; Ritter et al., 2001; Muthoni et al., 2017). In addition, rooted sprouts, previously separated from the nuclear seed tubers, can be used as starting material (Broćić et al. 2019b, 2022; da Silva Filho et al., 2020).

This study aimed to evaluate the effects of the cultivar and origin of planting material on the production of pre-basic seed potatoes (virus-free potato minitubers) in an aeroponic facility in Serbia.

Material and Methods

The experiment was conducted in 2019 (May - November) at the aeroponic facility in Guča, Serbia (Figure 1). Two potato cultivars, Sinora and Agria, were used in the research. Potato *in vitro* cultures were established from surface-sterilized tuber sprouts as described by Momčilović et al. (2014). Microplants were grown in a climate-controlled room (21 ºC, 16 h photoperiod, light flux 90 μmol m^{-2} s^{-1}) and were subcultured every 30 days by single-node stem cuttings. Before planting in aeroponics, microplants were planted in the substrate of perlite and sand (1:4) and acclimated in the greenhouse for 25 days. Sprouted minitubers, obtained as an aeroponic crop in a previous season, were also planted in the perlite and sand substrate (1:4). Plants from both sources were regularly watered and treated with a nutrient solution. After 20 days, plants developed from minitubers were transferred to aeroponics. The experiment in the aeroponic system started on May 28, 2019, organized in a complete randomized block design with 3 replications for each cultivar-plant origin combination and 10 plants per replica. The planting density in the aeroponic module was 24 plants per m^2 and the aeroponic system operated according to the fertigation regime described by Broćić et al. (2022). The minitubers were collected at ~ 14-day interval (from July to December), and the number of minitubers (tuber length ≥ 2 cm) per plant and minitubers mass were measured at the end of each harvest interval.

The total number of produced minitubers per plant, tuber mass and yield were quantified at the end of the cultivation period. The temperature was recorded in the aeroponic module and greenhouse during the experiment. Statistical analysis was performed using STATISTICA 12 (StatSoft, Inc. 1984-2014, Tulsa, OK, USA). The data regarding the number of minitubers per plant, the minituber's mass, and yield were analyzed using a two-factor analysis of variance (ANOVA) with plant origin and cultivar as the categorical predictors. The means were compared using Tukey's multiple comparison test. For the data analysis, the significance level $p < 0.05$ was used.

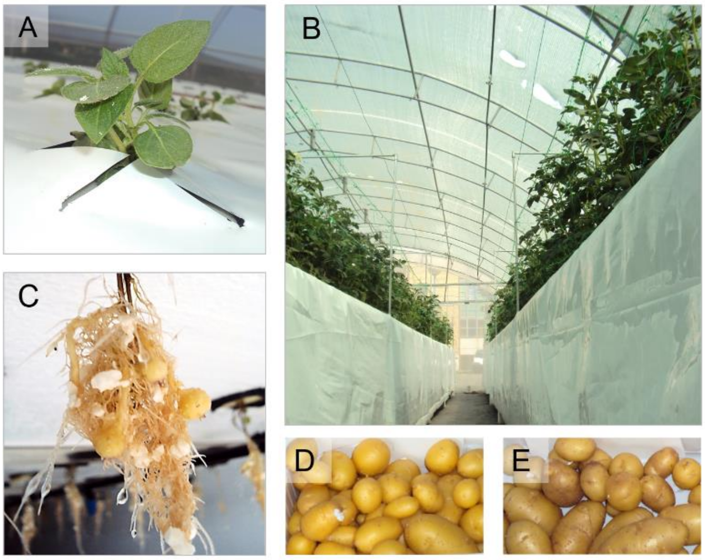

Figure 1. Cultivation of potato plants in the aeroponic system. (A) The foliage of potato plants after transfer to aeroponics. (B) Aeroponic facility for potato cultivation. (C) Belowground parts of potato plants in the aeroponic module at the beginning of tuber bulking. (D) Minitubers collected from aeroponically grown Agria plants. (E) Minitubers collected from aeroponically grown Sinora plants

Temperatures were measured in the aeroponic module (root zone) and greenhouse (haulm and leaves zone) of the aeroponic facility during the entire period of plant cultivation (Figure 2). The temperatures in the root zone of potato plants are especially important for the initiation and bulking of tubers, with the optimum for tuber initiation and tuber filling/growth in the 16-19 °C and 18-22 °C range, respectively (Broćić et al., 2019a). In the aeroponic module, daily average temperatures were significantly higher in the first half of the growing period (22.6 ºC) compared to the second part (16.6 ºC).

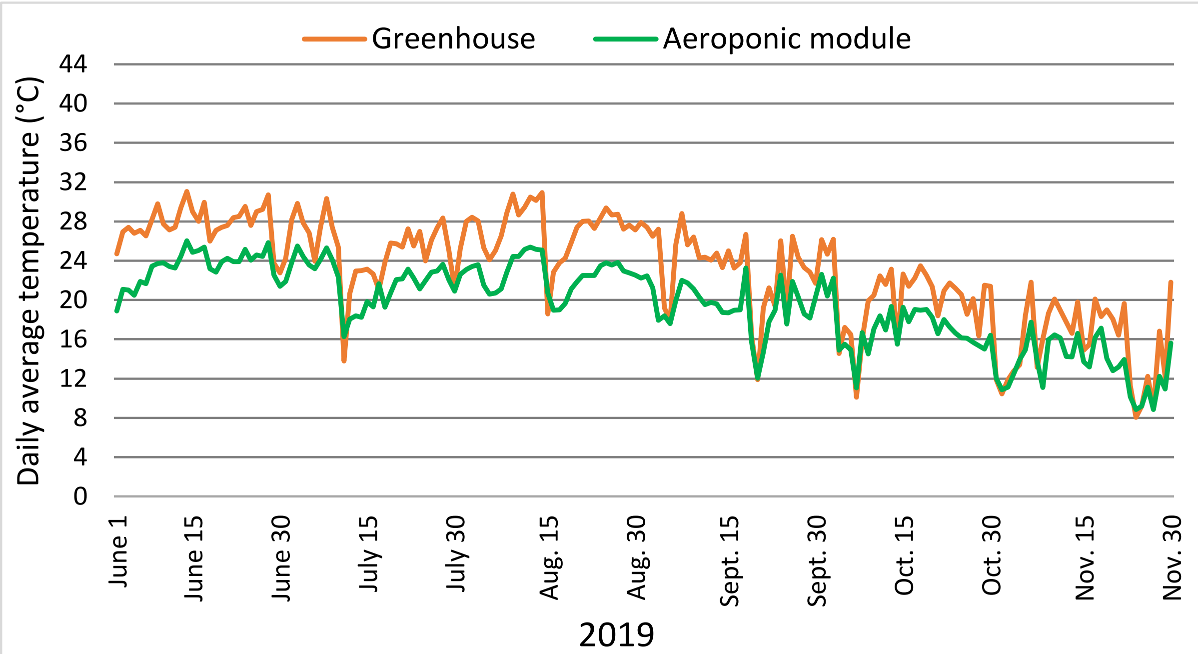

Figure 2. Temperature conditions in the aeroponic module and greenhouse during plant cultivation in 2019

Results and Discussion

Dynamics of Minituber Formation

Tuberization of Agria and Sinora plants of minituber origin started in July, 30-40 days after planting in the aeroponics. Agria plants steadily tuberized during the rest of the cultivation period (Figure 3A) and formed a significantly larger number of minitubers compared to cultivar Sinora which quickly completed the vegetative cycle (end of September). The highest number of minitubers was measured at the I-IV harvest in Sinora plants originating from minitubers and at the final harvest (X harvest) in Agria plants of the same origin (Figure 3A).

In general, a higher number of minitubers per harvest was collected from Sinora plants during the first part of the cultivation period and from Agria plants during the second half of the growing period. Sinora is an early maturing cultivar (85-90 days in open field production in Serbia), and it seems that aeroponic cultivation did not affect this developmental trait. On the other hand, Agria is a late maturing cultivar (120–135 days in open field production in Serbia) that was tuberizing during July – November, producing more tubers during the second half of the period when temperatures were closer to an optimum range for tuber initiation.

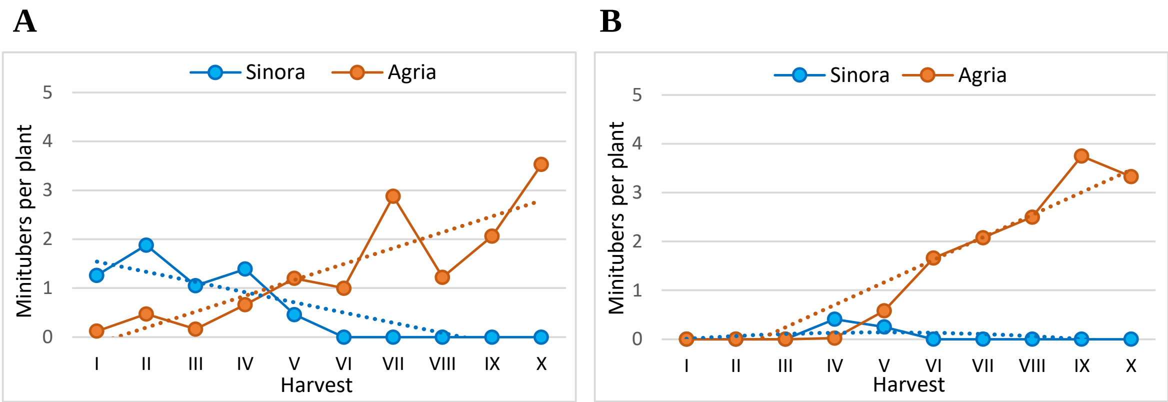

Figure 3. The dinamics of minituber formation for two potato cultivars grown in the aeroponic system. (A) The number of formed and collected minitubers per plant of minituber origin; (B) The number of minitubers per plant of *in vitro* origin

Concerning plants of *in vitro* origin, the first minitubers of Sinora and Agria were collected at the end of August (IV harvest). Rates of minituber formation were minor in Sinora plants, and the final number of minitubers was reached at the V harvest (Figure 3B). In Agria, the highest number of minitubers per harvest was collected at the end of the cultivation period (IX-X harvest). The heaviest tubers formed by Sinora plants of minituber origin (Figure 4A) were recorded at the beginning of the cultivation period (I and II harvest), while the lightest were measured at the harvest in mid-September (V harvest).

Considering Sinora plants of *in vitro* origin, masses of minitubers were minor, and only two harvests were conducted in the middle of the cultivation period (Figure 4B). The highest masses of Agria minitubers, produced by plants of both origins (Figure 4A and B), were measured in the middle of the cultivation period (IV-VI harvest). A decrease in the mass of Agria minitubers was observed in later harvests (the second part of the cultivation period) when daily average temperatures were favourable for tuber initiation, but not optimal for tuber bulking.

Nevertheless, the tuber mass in the later harvests was mostly above 8 g, which is considered by Farran and Mingo-Castel (2006) as sufficient for further usage as planting material.

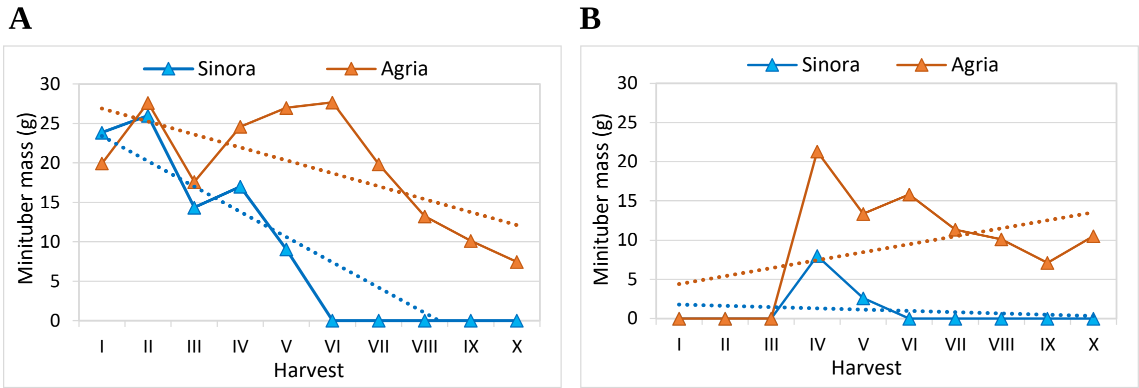

Figure 4. Tuber mass at harvest of two potato cultivars grown in the aeroponic system. (**A**) Minituber mass per harvest – plants of minituber origin; (**B**) Minituber mass per harvest – plants of *in vitro* origin

Effects of cultivar and plant origin on minituber production

Results of the two-way ANOVA revealed that both investigated factors, cultivar and plant origin, significantly affect the aeroponic production of minitubers (Table 1).

Table 1**.** Two-way ANOVA for potato minituber production in an aeroponic system

Parameter	**Factor**	**df**	**SS**	**MS**	**F**	***p***	**Sig.**
Number of minitubers per plant	Cultivar	1	307.446	307.446	137.889	2.53×10^{-6}	***
	Plant origin	1	15.098	15.098	6.771	3.15×10^{-2}	*
	Cultivar x Plant origin	1	24.596	24.596	11.031	1.05×10^{-2}	*
Minituber mass	Cultivar	1	59.541	59.541	29.108	6.50×10^{-4}	***
	Plant origin	1	285.480	285.480	139.562	2.42×10^{-6}	***
	Cultivar x Plant origin	1	27.816	27.816	13.598	6.15×10^{-3}	**
Yield per m^2	Cultivar	1	45.107	45.107	85.806	1.50×10^{-5}	***
	Plant origin	1	15.489	15.489	29.464	6.25×10^{-4}	***
	Cultivar x Plant origin	1	0.205	0.205	0.391	5.49×10^{-1}	-

Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The investigated factors affected the total number of minitubers formed per potato plant. A significant two-way interaction of cultivar: plant origin (Table 1) indicated that the effect of plant origin differs between the cultivars. The largest number of minitubers per plant (13.92) was recorded in the Agria plants of *in vitro* origin, while the lowest number was registered in Sinora (0.93) of the same origin (Figure 5A). The post hoc analysis (multiple group comparison) revealed that the plant origin did not significantly affect the number of formed minitubers in Agria. This concurs with our previous findings for this cultivar obtained in the 2018 season (Broćić et al., 2019b). Conversely, plant origin significantly influenced the number of tubers produced by Sinora plants. Sinora plants of minituber origin formed a significantly larger number of minitubers than plants of *in vitro* origin (Figure 5A).

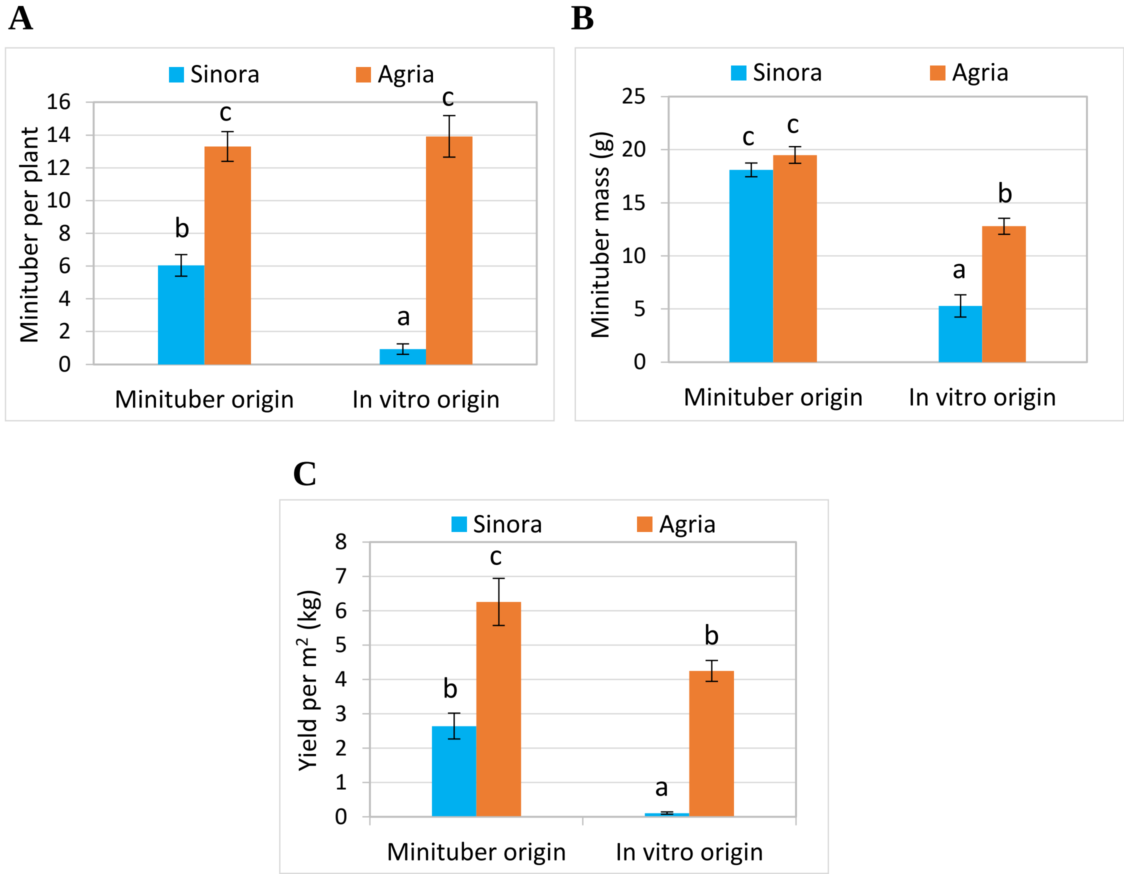

Figure 5. Effects of cultivar and plant origin on potato minituber production in aeroponic system. (A) The total number of minitubers formed per plant, (B) minituber fresh mass, and (C) yield per m^2 (24 plants) were quantified at the end of the cultivation period

The average mass of minitubers of two investigated cultivars was significantly higher in the plants originating from minitubers compared to plants of *in vitro* origin (Figure 5B). A significant two-way interaction of cultivar: plant origin (Table 1) indicated that the effect of factor cultivar differs between the plants of different origins. Indeed, Agria and Sinora plants of minituber origin produced tubers of similar mass, while Agria plants of *in vitro* origin produced significantly heavier tubers than Sinora. The yield was affected by the investigated factors of cultivar and plant origin (Table 1). Agria and Sinora plants of minituber origin gave a higher yield per m^2 than plants of *in vitro* origin (Figure 5C). The highest yield of minitubers (6.26 kg m^{-2}) was recorded in the Agria plants originating from minitubers, while the lowest yield was registered in Sinora of *in vitro* plant origin (0.11 kg m^{-2}).

Conclusion

The results of our study revealed that factors: plant origin and potato genotype affect the process of tuberization and, subsequently, minituber yield in aeroponically grown potato. In general, minituber-originated plants of both cultivars produced heavier tubers and gave better yields than plants of *in vitro* origin. However, Sinora plants of both origins quickly completed the growth cycle and had significantly lower yields than Agria plants, which indicates that it is not quite suitable cultivar for aeroponic cultivation.

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Phosphorus content and stratification in the soil layer 0-30 cm in conventional (CT) and no-till (NT) tillage systems long term experiment

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Abstract

Phosphorus in the soil is an essential nutrient less mobile than nitrogen and there is a need to determine its spatial variability. Attention is also paid to its loss because it is a pollutant of the environment. The aim of the study was to determine the patterns of phosphorus (P) distribution in the soil, stratification, after ten years of no-tillage (NT) system compared to the conventional tillage system, moldboard plowing (CT). The tillage systems were fertilized with the same amount of NPK fertilizers and urea and tillage was done on a conventional system (CT). The crop and sowing date are the same. Sampling was done with a soil probe sampling kit Eijkelkamp 04.01.SB. A soil layer of up to 30 cm (in 10 cm intervals) was sampled and analyzed in the laboratory. The soil was air-dried and ground, and in each of the samples the content of readily avaliable P (as P_2O_5) determined by the AL method according to Egner-Riehm (spectrophotometrically). The obtained values of readily available P in the NT system in the layer 0-10 cm are 32.79 mg/100g which is the highest in relation to the layers 10-20 cm - 25.54 mg/100g and 20-30 cm - 18.89 mg/100g and the values of same layers at CT. There is no such pronounced stratification and the values are 23.52, 23.21 and 22.55 mg/100g per layer in CT. The results of the study show a pronounced vertical stratification of P in the no-till (NT) system. The results show the need to raise the phosphorus level above the maximum value of the optimal content, above 25 mg/100g, in order to create a reserve before changing the conventional (CT) to the no-till (NT) system.

Key words: conventional tillage, no-till, phosphorus, stratification

Introduction

Intensive agricultural production today has much more noticeable social, ecological and economic implications than ever before due to increasing concerns about the sustainability of agriculture from the aspect of environmental protection. Therefore, soil cultivation, as a basic and important component of agricultural production technology, affects the sustainability of agriculture through its effects both on the processes taking place in the soil and on the properties and growth of crops (Khursheed et al., 2019). Tillage and fertilizer application change the soil profile in terms of nutrient distribution, thereby affecting the availability, adsorption, leaching, decomposition and mineralization of nutrients (Vilakazi et al., 2022). Conventional tillage (CT) practices have led to soil, water and nutrient losses on arable land and degraded soils with low organic matter content and fragile physical structure, which in turn has led to low crop yields and low water and fertilizer use efficiency. Therefore, it is essential to choose a tillage practice that maintains the physical properties of the soil needed for the successful growth of agricultural crops. In this sense, soil conservation tillage, in its many and varied forms such as zero tillage or no tillage (NT), protective tillage or mulch tillage, etc., promises the sustainability of agricultural productivity in positive correlation with the preservation of the environment (Khursheed et al., 2019). Conservation tillage methods, which are characterized by minimal mixing of layers and disturbance of the soil structure, improve the physical properties of the soil by reducing compaction, soil erosion, surface water runoff and the risk of aquifers (Nze Memiaghe et al,. 2022). They also reduce production costs (Khursheed et al., 2019). The adoption of no-tillage (NT) production systems resulted in uniquely different soil phosphorus (P) distribution patterns compared to conventional tillage (CT) systems (Jones et al., 2007). Consequently, P availability, and therefore fertilizer P requirements, may be different in NT than in CT systems. Sustainable management of available phosphorus in the soil implies a balanced application of fertilizers, i.e. the balance between the supply of P (source) and the actual need of crops for phosphorus from the soil (Nze Memiaghe et al,. 2022). To achieve this goal, it is important to increase our knowledge of the spatial variability of soil phosphorus at NT to develop more rational and cost-effective P fertilization programs that will increase yields and reduce P losses to the environment. Therefore, the objectives of this research were to determine the difference in vertical P distribution patterns between NT and CT systems.

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Material and Methods

An experiment with tillage systems was established in 2006 with 2 tillage systems on 3 fields of 1.25 ha each. Zone tillage or strip tillage (ST) with two ways of applying mineral fertilizers in basic fertilization: the first field application with cultivation – banding in strip (ST1) and the second field application on the surface and then cultivation (ST2). For strip till we used own Case DMI 5310 with Gandy box for dry fertilizers (Fig. 1.) addition of the implement photo would much help the reader to understand what was it. Third field is conventional tillage or moldboard plowing (CT) with surface applied mineral fertilizers before tillage. In the autumn of 2008, a third tillage system was introduced, the mulch tillage (MT), where the basic application of mineral fertilizers was done on the surface than tilled. Since 2014, the field of zone tillage (ST2) has been transferred to the fourth tillage system no tillage (NT) (Molnar et al., 1999). The total size of the experiment is 5 ha where the tillage system is always the same, and where the following cultivated plant species are: winter wheat, corn, sunflower, soy and canola.

Figure 1. Strip till equipment Case DMI 5310 with dry fertilizer box

The basic fertilization in the fall of 2021 is as follows: NPK 8-16-24 mineral fertilizer was spread over the surface in the amount of 250 kg/ha on both tillage systems. On the conventional tillage (CT) plowing was done in December 2021 and on the no-tillage (NT) the applied mineral fertilizer remained on the surface. On CT an NT before planting we applied nitrogen fertilizer urea in the amount of 200 kg/ha and finished seed bad preparation of soil and incorporation of urea fertilizer on CT in the same day. On the no-tillage (NT) urea remained on the residues and surface. The sowing was done ten days later on April 27th 2022 at sowing density 66511 kernels/m^2, i.e. 70 x 21.5 cm.

After sowing, soil samples were taken in two tillage systems. The samples were taken at three locations in the system of conventional tillage (CT) and no tillage (NT) at depths of 0-10, 10-

20 and 20-30 cm. For sampling we used a one-piece tubular probe for sampling in solid soils up to a depth of 1m from the company Eijkelkamp (04.01.SB). Stamping was done with a steel hammer with plastic heads, which is an integral part of the kit. The place of sampling in both systems was between the rows due to the method of application of mineral fertilizers.

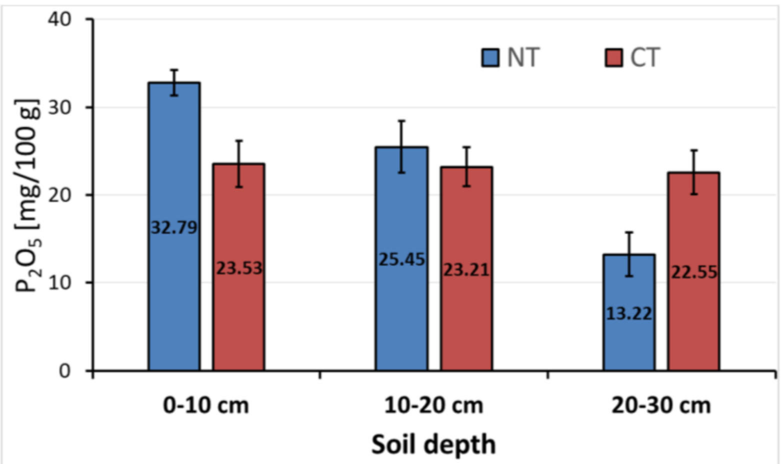
Both tillage systems are based on soil type: chernozem, subtype: on loess and loess-like sediments, variety: carbonate, form: medium depth. Texture: clay loam. In the sampled and analyzed layer 0-30 cm, the ratio of sand, silt and clay is 45.4% : 31.12% : 22.3%.

The soil was air-dried and ground, and in each of the samples the content of readily avaliable P (as P_2O_5) determined by the AL method according to Egner-Riehm (spectrophoto-metrically).

Results and Discussion

Patterns of distribution of phosphorus (P) in soil under a long-term conservation system without tillage (NT) as well as under conditions of conventional tillage with field plow (CT) were discussed on the basis of laboratory analysis of readily available phosphorus content.

The mean value of the concentration of readily available P was significantly higher in the 0-10 cm layer with the NT treatment system 32.79 mg/100g compared to the same layer with CT with 23.52 mg/100g. In the second layer 10-20 cm the values were close: for NT 25.54 and for CT 23.21 mg/100g. The content of readily available phosphorus is higher in the 20-30 cm layer at CT of 22.55 compared to NT 18.89 mg/100g (Graph. 1).



Graph. 1. The mean values of readiliy available phosphorus by depth depending on the tillage system, conventional tillage (CT) and no-tillage (NT) (mg/100g)

Also, the mean values of readily available P content were higher in the 0–10 cm soil layer compared to 10–20 cm and 20–30 cm in the NT system. In contrast, mean values were similar for all three soil layers in the CT system. Our results show that P stratification exists by soil depth shown in the graph. 1.

High content of avaliable P in first soil layer of NT is no disturbance of the soil over the entire surface, but only in the seed laying zone, which is up to 5 cm wide (Molnar et al., 1999)[8]. After the basic processing in CT, plowing with a moldboard plow, the fertilizer spread from the surface of the soil as well as the harvest residues are distributed in deeper layers, which is shown by the obtained value of the P_2O_5 content. Many studies (Abdi et al., 2014, Cade-Menun et al., 2010, Bertol et al., 2007, Duiker & Beegle, 2006, Messiga et al., 2012, Rodrigues et al., 2016) found that P stratification is more pronounced in the soil in NT systems compared to CT. In this research, soil P stratification can be explained by crop residues and lack of soil-fertilizer mixing according to the research (Nze Memiaghe et al. 2022).

In order to analyze the influence of soil depth and tillage system on the phosphorus content in the soil, a two way ANOVA was carried out using the program IBM SPSS Statistics, Trial Version 22.0. Multiple comparisons of mean values of factor variants were conducted using the LSD test at two levels of statistical significance (1% and 5%).

Two-way ANOVA shows that the variation of phosphorus content is significantly influenced by the analyzed factors. Namely, the soil depth factor has the greatest influence on the phosphorus variation ($p<0.01$), while the processing system factor has a smaller share in the variation, but statistically significant at the 5% significance level ($p<0.05$). The interaction of soil depth and tillage system has a statistically highly significant ($p<0.01$) contribution to phosphorus variation (Table 1).

Table 1. Two-factor analysis of variance for phosphorus content at different soil depths and in different tillage systems

Sources of variation	Sum of squares (SS)	Degrees of freedom (Df)	Mean of squares (MS)	F - value	p - value
Soil depth (A)	165.884	2	82.942	14.269**	0.001
Tillage system (B)	30.681	1	30.681	5.278*	0.040
A × B	125.575	2	62.787	10.802**	0.002
Error	69.752	12	5.813		
Total	391.892	17			

The LSD test was applied in order to make a multiple comparison of mean values of phosphorus content in the analyzed treatments (Table 2).

Table 2. Mean values of phosphorus content (mg/100 g of soil) at different soil depths and in different tillage systems

Soil depth (A)	Soil tillage systems (B)		Average
	No till	Conventional tillage	
0 – 10 cm	32.79	23.53	28.16
10 – 20 cm	25.45	23.21	24.33
20 – 30 cm	18.89	22.55	20.72
Average	25.71	23.09	24.40
LSD test			
	Soil depth (A)	Tillage systems (B)	A × B
$LSD_{0,05}$	4.289	2.502	6.065
$LSD_{0,01}$	6.013	3.910	8.503

The highest value of phosphorus content was found at a depth of 0-10 cm (28.16 mg/100 g) and statistically highly significant ($p<0.01$) differs from the value at a depth of 20-30 cm (20.72 mg/ 100 g). On average, for all soil depths, a significantly higher ($p<0.05$) value of phosphorus content was found in the NT compared to the value measured in the CT system (25.71, i.e. 23.09 mg/100 g). The interaction of soil depth × tillage system also had a significant effect on phosphorus variation. The highest value of phosphorus content was found at a depth of 0 - 10 cm in the NT (32.79 mg/100 g), while the lowest value was found at a soil depth of 20 - 30 cm, also in NT (18.89 mg/100 g). These differencies between first and third layer in NT have been comfirmed by other researches (Nze Memiaghe et al,. 2022). Observing the phosphorus values at all depths and tillage systems, it is concluded that the phosphorus content at a depth of 20-30 cm is higher in the CT system than in the NT, while at all other soil depths the phosphorus content is higher in to the NT compared to the content of the same in the CT (Table 2).

In NT, this still high content P_2O_5 in 10-20 cm layer is most likely the consequence of decades of plowing and the use of NPK fertilizers without analysis and appropriate recommendations (Deubel et al., 2011). We can say that depending on precipitation, temperature, activity of microorganisms and the presence of air pores, this phosphorus is available to plants.

Content in NT 20-30 cm layer shows that in this layer, phosphorus is more intensively used by crops and other organisms (18.89 mg/100g). The properties of phosphorus are such that it is not possible to move it from the second layer and increase its content in the third layer.

In the case of the CT system there was no significant differencies compared first and third layer (according Muukkonen et al., 2007, Lupwayi et al., 2006). This speaks in favor of the characteristics of the use of the moldboard plow in soil cultivation and its mixing of soil layers as well as what is on the surface to the depth of cultivation.

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Conclusion

In both tillage systems, NT and CT in the last 10 years, the amount of applied P by fertilizer for cultivated crops was identical in quantity (kg/ha), but the P amount depended on the planned crop yield. On the NT system, the applied fertilizer remained on the surface since the beginning of the trial in 2006 which resulted in the highest content of readly avaliable P in first layer (0-10 cm) 32.79 mg/100g compared with other layers (10-20 and 20-30 cm) in NT and all same layers in CT. In the same time NT third layer (20-30 cm) have the lowest avaliable P content of 18.89 mg/100g compare to all layers in CT. The results of the study show a pronounced vertical stratification of P in the no-till (NT) system. The interaction of soil depth and tillage system has a statistically highly significant ($p<0.01$) contribution to phosphorus variation.

There is no significant differences of P content between layers in CT. The available P content are 23.52, 23.21 and 22.55 mg/100g.

The results show the need to raise the phosphorus level above the maximum value of the optimal content of 25 mg/100g in order to create a reserve before changing the conventional (CT) to the no-till (NT) system.

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Low-input farming for agricultural sustainability

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Abstract

The aim of this paper is to characterize the low-input farming considering the resources it uses, scale of farming operations to which it suits, and its environmental impacts along with sustainability issues. The research was conducted by literature review. Low input farming systems seek to optimize the management and use of on-farm resources and to minimize the use of production inputs as off-farm resources, such as purchased fossil fuels, chemical fertilizers, and pesticides. The research has shown that low-input farming can contribute to lessening the use of synthetic pesticides, fertilizers, and fossil fuels while enabling the recovery of soils' fertility, and biodiversity in the agroecosystems. Thanks to the expected recovery of soil fetility and porosity, there is anticipated greater resilience of low-input farming to extreme climatic events like droughts when compared with currently prevailing intensive farming. Therefore, the low-input farming can be much appreciated among the farming options for the improved sustainability.

Key words: low-input farming, soil fertility, pesticide emmissions, biodiversity, resilience

Introduction

Agriculture still provides the essential goods to humanity, including the majority of food, and some fiber and energy. However, at the same time it pollutes the ecosystems (by the use of synthetic fertilizers and by pesticides emmissions), causes biodiversity loss (by spread of arable farming and pesticides emmissions), loss of soil fertility (loss of soils' productive capacity by excessive use of mineral fertilizers, loss of humus content and soil compaction by heavy machinery), and contributes to the exhaustion of fossil resources (crude oil, natural gas and phosphate rocks) and carbon (CO_2) emmissions to the atmosphere (due to diesel fuel combustion, humus loss in arable soils and gas combustion in nitrogen fertilizers production). Sustainable development of humanity largely depends on the sustainability of agriculture, as well as on its impacts on ecosystems.

There is no doubt that chemization and mechanization of agriculture were associated with a great increase in food production and productivity of human labor (machines have largely replaced the labor), but in the view of contemporary threats to the sustainability, they have to be re-thinked. Besides the chemical-, machinery- and capital-intensive agriculture, during the last several decades there emerged some more sustainable options, including the integrated agriculture, organic agriculture, ecological agriculture, regenerative agriculture, permaculture and biodynamic agriculture. Besides the mentioned alternatives, there is general growth of interest for the low-input farming.

According to Poux (2007), low input farming systems should be considered as a core option for Europe. Low Input Farming Systems seek to optimize the management and use of on-farm resources and to minimize the use of production inputs as off-farm resources, such as purchased fossil fuels, chemical fertilizers, and pesticides (Parr et al., 1990; cit. Poux, 2007). Main advantages of low input farming systems over the high input ones are found to be the potentialy higher efficiency at the farm level, reduction of pollution risks and beneficial effects to biodiversity and lanscape (Poux, 2007). According to Garré (2022), the inclusion of work horses in sustainable transitions, can help increase the farm autonomy and sustainability of European smallholdings. Since the animal-drawn agronomy relies on the on-farm produced fodder as a source of clean and carbon-neutral bioenergy (Gantner et al., 2014), it perfectly fits into concepts of low-input farming systems and circular economy as well.

In shaping the future options for improved sustainability, there might be beneficial to consider the nowadays almost neglected traditional knowledge. Namely, indigenous and other long-resident peoples, have accumulated the knowledge, that has enabled them to live in one place, using the resources of their homelands sustainably, for countless generations (Turner et al., 2022).

On-farm produced soil fertilizers for low-input farming

Besides the main goals for which the livestock is reared (milk production, meat production, hides and fur production, work), there is at the same time unavoidable production of farmyard manure (FYM). FYM is recognized as a valuable resource for rising the soils' fertility and crops' yields, from the ancient times till the nowadays (Singh et al., 2020). According to the same group of authors, large-scale intensive farming has resulted in several physical and physiological problems in the soil and is also responsible for soil and environmental pollution. There is excess of chemical fertilizers in the unavailable form that are left in the soil, and these residuals cannot be absorbed by the plant. In the case of rainfall soon after application of chemical fertilizers, they come washed away and accumulated in water bodies and cause water pollution, resulting in algal bloom. Application of mineral N-fertilizers is proven to decrease the organic matter content in soils, thus degrading the soil fertility (Mulvaney et al., 2009). Contrary, rising the soils' fertility by FYM application and green manuring is known to improve chemical and physical properties of soils including the reduction of bulk density and increase of soil porosity and aeration (Singh et al., 2020), what is associated with increased rainwater infiltration rate. Increase of soils' porosity, water infiltration rate and water holding capacity becomes crucially important for mitigation of drought effects (because soil acts as a sponge for water storage), and therefore it is expected that the organic soil fertilization will rise its appreciation by farmers and conscient consumers. Annual production and quality of FYM varies with the livestock species, age, diet, productivity, use of bedding and storage. In the research of Ambrušec et al. (2021), green manuring with crimson clover was found competitive with chemical fertilizers for achieving the desired maize yields, but green manuring was associated with the need for additional field operations (i.e. energy consumption) for establishing the green manure crop and for incorporation of its herbage into soil. Therefore Ambrušec et al. (2021) proposed to consider the use of animal traction fuelled by the on-farm produced fodder in order to avoid the additional monetary expences for purchased fuel.

Soil compaction is one of the major problems faced by modern agriculture (Hamza and Anderson, 2005) since it threatens many determinants of soil fertility (Soane and Ouwerkerk, 1995; Horn et al., 1995). It is well documented that one of the main causes for soil compaction is the overuse of machinery (Hamza and Anderson, 2005). Oppositely, horses provide a tried and tested solution to prevent soil compaction (Herold et al., 2009; cit. Gantner et al., 2014). Although horses can under certain circumstances impose a higher ground pressure than tractor tyres, the compaction effect is limited to the top few centimetres of the soil profile because of the comparatively lower weight (Wyss, 1999; cit. Gantner et al., 2014). Therefore, there is anticipated the restoration of soil pores required for rainwater infiltration and accumulation for the drought periods, by reintroduction of animal traction into field works, what would enhance the adaptation to the forthcoming whether extremes, including droughts.

Findings of Jackson (1988) support the thesis that low-input farming style (Amish) is associated with greater soil fertility and water infiltration rate than in conventional farming (Table 1).

Table 1. Soil fertility parameters of two neighbouring farms: Amish and conventional no-till in Holmes County (Ohio, USA; Jackson, 1988)

Soil fertility parameter	Amish farm	No-till farm
Cathion exchange compley (meq)	7.5 +/- 0.8	7.0 +/- 2.0
Base saturation Ca^{2+} (meq)	67.0 +/- 12	50.0 +/- 19.0
Base saturation Mg^{2+} (meq)	12.0 +/- 3.0	8.0 +/- 4.0
pH	6.38	5.65
Organic matter (%)	2.6 +/- 0.3	1.9 +/- 0.1
Bulk density in maize fields (g/ccm)	1.29 +/- 0.06	1.5 +/- 0.01
Steady state infiltrability in maize fields (ml/hr)	6.88	0.93

On-farm produced plant protection preparations for low-input farming

Synthetic pesticides have provided very efficient crop protection from various pests since the Green Revolution. However, there are rising problems of emmissions of toxic compounds into environment and loss of biodiversity associated with the excessive use of synthetic pesticides. Another arising problem is the loss of efficiency of these pesticides due to achieved resistants of pests. All above mentioned pushes the researchers and practical farmers to search for more sustainable options. Luckily, many plant-derived and natural preparations have shown the more or less acceptable efficiency in controlling the losses caused by insect pests (Ignacimuthu, 2012) and fungal pathogens (FAO, 2015), and they can be prepared on-farm. In this way the reliance on the on-farm resources would help to lessen the emmissions of toxic substances into environment, while providing the safe food free of toxic residues. Moreover, this rises the autonomy and resilience of farmers what might be very advantegous in circumstances of disturbed supply chains. However, the use of on-farm produced resources for plant protection is more convenient for small farmers than for large agricultural enterprises since it requires much labor for their preparation. And, there should also be pointed, that the research of the on-farm produced plant-protection and pest-control agents has to be continued in order to find more efficient methods and preparations. Collection of knowledge from the old native and indigenous people (even in Europe) can much help in getting the ideas for scientific testing, as already noted by the authors (not published data about preparation and effectivity of traditional herbal brews for plant pests control). The research of assisting the predatory birds activity for the control of field rodents was inspired by the told memories of old farmers of eastern Croatia (Simunić et al., 2020).

Lessening the consumption of energy from fossil resources and reducing the CO_2 emmissions

Lessening or even avoidance of the consumption of energy from fossil resources in crop production can be achieved by partial or even complete substitution of mechanical tractors with the animal traction (e.g. with draft horses), thus also reducing the CO_2 emmissions to the atmosphere and achieving the farmers' self-reliance and improved resilience in cases of disturbed supply chains. Namely, all the fodder consumed by draft animals can be produced on-farm, and it is produced by the recent photosynthesis (i.e. recent binding of the atmospheric CO_2 into organic compounds comprised in fodder; Gantner et al., 2014). Vegetable, vine and orchard/fruit farmers are more likely to accept the animal work because thay already employ much labor, despite the modern extensive mechanization in cereals, oilseeds and pulses production. A greater hesitancy is expected for farmers that produce cereals, oilseeds and pulse grains because the harvest without diesel-powered combine-harvesters is hard and excessive labor (namely, the mowing and separation of grains from the straw), unless there are being used mehanized solutions driven by animal force. However, modern Amish communities that adhere strictly to solely animal and human power have proven that all the operations in food production still can be done without the fossil fuel powered machinery. Despite the shown feasibility and attractivity of animal work in agriculture, according to the authors' knowledge, experienced people who can train and efficiently use the draft horses in agricultural work are rare in most of the modern societies (not published data). In Croatia, this traditional knowledge can be collected from the little number of old people, and can be transmited only through the direct in-person teaching and practical work, thus proving the importance of long-resident peoples as stated by Turner et al. (2022). On the other hand, there are emerging professional associations that collect knowledge on the training and use of animal work, improve the welfare of draft animals and develop the improved implements for greater work efficiency and more comfort both to animals and men (Schmit et al., 2022).

Saving the fossil energy and lessening the CO_2 emmissions in livestock production can be achieved by increased reliance on grazing instead of feeding the stored forages. Namely, for harvested perennial forages (lucerne hay and haylage, grass-clover mixes hay and silage, meadow hay and silage) the greatest part of total production costs, as well as of the used energy is associated with mowing, spreading, collecting, baling and transporting of harvested herbage. Also, considering the maize for the whole-crop silage (which is currently the most important annual forage crop), much fossil fuel is being consumed for soil preparation, crop establishment, and cultivation each year, besides for the harvesting. In the case of grazing, all mentioned jobs are omitted. Namely, the livestock does all job instead of us. Thus, not only the diesel fuel is being saved, but also the CO_2 emmissions are reduced. However, if there is aimed to maintain the constant output of livestock products, with shift to the grazing, there has to be increased number of livestock and increased pasture area due to lesser per-head productivity of grazed livestock when compared to TMR fed ones. Increased pasture area can be obtained either by establishing leys on arable soil or by spread of livestock to the currently much abandoned seminatural grasslands of WB-countries, or both. Establishing short- or mid-term grazed leys (2-5 years) on arable soils will likely improve the overall fertility of already degraded arable soils by increase of the soil organic matter content and recovery of soil porosity, while simultaneously enabling for the improvement of biodiversity in agroecosystem (Gantner et al., 2022). Since the rise in soil organic matter is consequence of plant biomass growth and deposition (live roots and litter), it simultaneously sinks the carbon from atmosphere and sequesters it into the soil, thus fulfilling some of the main aims of current environmental policies.

Producers and consumers attitudes considering the low-input farming

According to the preliminary research (not published yet), farmers attitudes vary in a broad spectrum, from sympathies to the reluctance. Sympathies are mainly due to the expectations for improved resilience and self-reliance for low-input farmers, and due to improved overal sustainability of agriculture and societies. Reluctance stems from the anticipated much greater involvement of human labor that is needed for guiding the draft animals, handling the simple soil-tillage implements, making the on-farm preparations for crop protection, poor work efficiency (only small farms can be operated in this manner) and lack of convenience and comfort that modern tractors provide to farmers. If the low-input farming is to be a significant contributor to the sustainable development of agriculture and societies, along with other more sustainable farming options, there would be needed personal inner transformation of farmers and potential new farmers. In line with this are the findings of Woiwode et al. (2021) who stated that, besides the technological solutions, there would be required an inner transformation for system change towards sustainability. In this context, the inner transformation relates to consciousness, mindsets, values, worldviews, beliefs, spirituality and human–nature

connectedness. In order to inspire the changes considering the reluctant attitudes, there will be required upgraded education of all stakeholders in agri-food sector. Considering the consumers, they mainly show much sympathies but with some scepticism about the acceptance of low-input farming by modern farmers.
The old natives' wisdom that the land is not inherited from our ancestors but is borrowed from our descendants might be helpful in motivating the required transformational thinking.

Conclusion

The research has shown that low-input farming can contribute to lessening the use of synthetic pesticides, fertilizers, and fossil fuels while enabling the recovery of soils' fertility, and biodiversity in the agroecosystems. Thanks to the expected recovery of soil fetility and porosity, there is anticipated greater resilience of farming to extreme climatic events like droughts. Through the reliance predominantly on the on-farm produced resources, low-input farming is expected to contribute to self-reliance of farmers and better tolerance of the supply-chain disturbances. Besides the expected lesser emmissions of toxic compounds and CO_2, there is also expected greater sequestration of atmospheric CO_2 into soil organic matter due to employment of organic soil fertilization and livestock grazing in low-input farms. Due to all bove mentioned, the low-input farming can be much appreciated among the farming options for the improved sustainability.

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“Poljoprivreda s niskim ulaganjima” za poboljšanu održivost poljoprivrede

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Sažetak

Cilj ovog rada je okarakterizirati poljoprivredu s niskim ulaganjima s obzirom na resurse koje koristi, opseg poljoprivrednih poduzeća kojima odgovara i njezin utjecaj na okoliš zajedno s pitanjima održivosti. Poljoprivreda s niskim ulaganjima nastoji optimizirati korištenje reursa porijeklom s farme, a minimizirati korištenje resursa porijeklom izvan farme, kao što su kupljena fosilna goriva, kemijska gnojiva i pesticidi. Istraživanje je provedeno pregledom literature. Istraživanje je pokazalo da poljoprivreda s niskim ulaganjima može pridonijeti smanjenju upotrebe sintetičkih pesticida, gnojiva i fosilnih goriva, a istovremeno omogućiti oporavak plodnosti tla i bioraznolikosti u agroekosustavima. Zahvaljujući očekivanom oporavku plodnosti i poroznosti tla, očekuje se veća otpornost poljoprivrede s niskim ulaganjima na ekstremne klimatske događaje poput suša u usporedbi s trenutno prevladavajućom intenzivnom poljoprivredom. Zbog svega navedenog, poljoprivreda s niskim ulaganjima može biti vrlo cijenjena među poljoprivrednim opcijama za poboljšanu održivost.

Ključne riječi: poljoprivreda s malim ulaganjima, plodnost tla, emisije pesticida, bioraznolikost, otpornost

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Energy equipment with tractors in the cooperative "Agroprom"

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Abstract

The agricultural cooperative "Agroprom" in Stara Pazova was audited to assess their agricultural machinery equipment. The article presents the results of the audit of agricultural machinery equipment of the agricultural cooperative "Agroprom" from Stara Pazova. The cooperative cultivates 2,592 ha with 35 two-axle tractors and 79 implements. In terms of the number of tractors, John Deere is the most represented with 15 units or 42.86%, while Landini Ghibli and IMT-542 are the least represented with 1 unit each or 2.86% of the total number of tractors. The total available engine power of all tractors is 3,655.0 kW, of which John Deere tractors account for more than half, 51.78% or 1,877.78 kW. The specific load of tractors on the cultivated area is 0.71 ha/kW. There are 1.31 tractors per 100 ha of cultivated area. The energy equipment of the cooperative is expressed on average by the nominal engine power of the two-axle tractors per unit area of 1.41 kW/ha. A tractor with an average engine power of 104.43 kW works 76.23 ha with 2.32 implements. The average age of tractors is less than 10 years. Most tractors are between 11 and 12 years old, 62.86%, and 5.71% of the total number of tractors are less than 5 years old. From the above data it can be concluded that the equipment of the agricultural cooperative "Agroprom" from Stara Pazova with modern agricultural machinery is at a satisfactory level.

Key words: cooperative, tractors, implements, power machinery, age of machinery

Introduction

In the rural areas of Serbia, agriculture is the main activity, carried out on a large scale by farms and family businesses. Individual production on farms cannot satisfy market demand for quantity, quality and continuity and in most cases is not economically competitive. One of the ways to better and more efficient organization of agricultural production is the association of producers in various forms, as well as a cooperative run by the producers themselves. The local initiative and organization of farmers contributes significantly to rural development. As one of the oldest forms of business, cooperatives are characterized by a variety of peculiarities that require a more detailed approach in their study and identification of the factors that determine their activity. In this way, agricultural producers obtain financial resources in a simpler and faster way after selling and marketing their products. The aim of the study is to determine the sowing structure, the number of tractors and implements, and based on this, to analyze the energy equipment and the workload of the machines with arable land (Veljković et al., 2020). The main activity of the cooperative is the production of agricultural products. It also deals with the purchase, storage and sale of agricultural products. The cooperative has a total of 34 two-axle tractors and one telehandler tractor for loading and transporting goods. The most represented are tractors of medium category - 28 or 80%, then heavy tractors - 6 or 17.14% and light tractors - 1 or 2.86% of the total number of tractors. The cooperative has 14 different types of tractors, produced by 9 different manufacturers.

Materials and Methods

The research was conducted in the southeastern part of Srem in the agricultural cooperative "Agroprom" from Stara Pazova (AP Vojvodina, Srem district). Based on the accounting records of the cooperative, the sowing structure of the land, the total number of tractors by category and manufacturer and the number, type and age of implements were determined. According to Nikolić et al. (2013), tractors were divided into four categories based on engine power and on this basis the representation was analyzed according to the number and power of tractor engines. Based on the engine power of each type of tractor, the total available rated power and the average power of the tractor engine were calculated. According to the cooperative energy system formula, the effective power of the tractor engine per unit of arable land (kW/ha) was calculated:

$$Et = \frac{\sum_{i=1-n}^{n} Pe(i)}{\sum_{i=1}^{m} Ai} (kW/ha),$$

Et - energy equipment with tractors in the cooperative,

A (ha) – agricultural area,

Pe (kW) – the effective power of the tractor engine and the number of tractors per 100 ha were calculated.

The number of attached machines per tractor was calculated based on the total number of machines and tractors (machine/tractor) and the cultivated area per attached machine (ha/machine). Data from the Statistical Institute of the Republic of Serbia were used for the study.

Results and Discussion

Within the cooperative "Agroprom" there are three farms in Stara Pazova, Golubinci and Popinci. The cooperative cultivates a total of 2,592 hectares, of which 340 hectares are owned by the cooperative and the rest of the land is owned by subcontractors who provide services with the available machinery. The cooperative generates a significant source of income by providing services to other agricultural producers. The income from services in the period from 2017 to 2019 was 90,846,463 RSD, 86,520,241 RSD or 50,558,104 RSD (Gavrilović et al., 2023). The cooperative employs 27 workers, 18 of them with higher education (III and IV), who work as operators of agricultural machinery, silo and warehouse workers. The same number of workers with higher and technical education and unskilled workers, 3 workers each, are also employed. In addition to the production of agricultural products, the cooperative also deals with the purchase, storage and sale of agricultural products. The cooperative owns a silo with a capacity of 16,000 tons, and the construction of another silo with a capacity of 8,000 tons is planned, as the existing facility is not sufficient to store the quantities produced. In addition, the cooperative owns a farm with a total area of 2 ha, where, in addition to the silo, there is also a covered area for storing machinery and some of the raw materials needed for production, such as seeds, fertilizers and pesticides (Gavrilović et al., 2023).

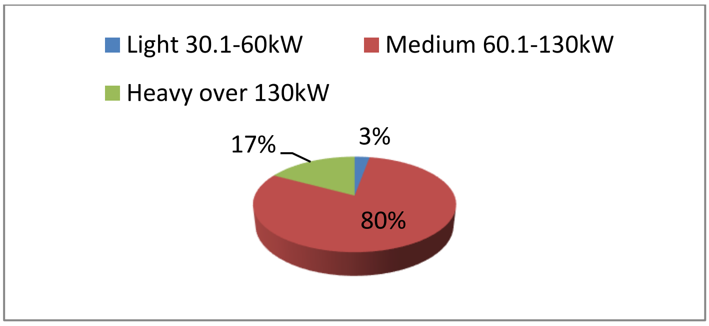
Table 1. Number and power of tractor engines by types and manufacturers

Serial number	Manufacturer and type of tractor	Number of pieces	Engine power (kW)	Total power (kW)	Participation by number condition (%)	Participation by engine power (%)	Total age of the tractor (years)
1.	IMT-542	1	31.30	31.10	2.86	0.85	12
2.	Lamborghini Premium	4	74.55	298.2	11.43	8,16	4x12=48
3.	Belarus MTZ	9	75.67	681.0	25.71	18.63	9x12=108
4.	John Deere	15	125.18	1,877.78	42.86	51.38	15x9=135
5.	Fendt 930	2	223.71	447.42	5.71	12,24	2x6 =12
6.	Case IH Maxxum 115A	2	85.76	171.52	5.71	4.69	2x6 =12
7.	Landini Ghibli 100	1	67.00	67.0	2.86	1.83	10
8.	Telehandler JCB 531-70	1	81.00	81.00	2.86	2.22	10
9.	Total/average	35	104.43	3,655.02	100	100	347/9,91

The cooperative has a total of 34 two-axle tractors and one telescopic tractor (Tab. 1) for loading and transporting goods and all tractors, except the IMT-542, are front- and rear-wheel drive. By the number of tractors, there are most John Deere tractors (John Deere 6620, 6630, 6530, 8330,8420) 15 units or 42.86%, then Belarus MTZ (MTZ-892, MTZ-1025) 9 or 25.71%, Lamborghini Premium (Lamborghini Premium 950 and 1060) 4 or 11.43%, Fendt 930 and Case IH Maxxum 2 units each or 5.71% and Landini Ghibli, IMT-542 and Telehandler JCB 1 unit each or 2.86% of the total number of tractors. The situation is similar when considering the energy share of the tractor in the total rated power of the engine tractor. More than half (51.78%) of the available tractor power of 3,655 kW is accounted for by John Deere tractors with 1,877.78 kW. They are followed by Belarusian MTZ tractors with a total of 681.0 kW or 18.63%, Fendt 447.42 kW or 12.24%, Lamborghini Premium 298.2 kW or 8.16%, Case IH Maxxum 171.52 kW and 4.69%. The tractor IMT-542, only 0.85% with 31.10 kW and Landini Ghibli 67 kW or 1.83% take the smallest share in the total tractor power. Authors Nikolić et al. (2013) divided tractors into 4 categories depending on engine power: Mini tractors up to 30 kW, light tractors from 30.1-60 kW , medium tractors from 60.1-130 kW and heavy tractors over 130 kW. According to this classification, 28 or 80% of tractors belong to the medium category, 6 or 17.14% of tractors belong to the heavy category, and 1 or 2.86% of tractors belong to the light category (Graph. 1). The cooperative has 14 different types of tractors from 9 different manufacturers. From the total number of tractors, only 10 (28.57%) are from the East (former SFRY countries and USSR), while the other 25 (71.43%) are from Western countries (Western Europe and the USA). In the Republic of Serbia, tractors of the power class 33-66 kW dominate in the farms of legal entities and entrepreneurs, followed by tractors of the power class above 89 kW and the power class 19-37 kW, which are now equally represented

(Božić et al., 2014).

The study of the age of the tractors showed encouraging results, as the tractors are on average less than 10 years old. Most tractors are between 11 and 12 years old (62.86%), followed by 3 tractors aged 10 years (8.57%), 8 tractors aged 6 and 7 years (22.86%) and the youngest are 2 tractors (5.71%) John Deere 6530 purchased in 2017. At the level of the Republic of Serbia, of the total number of two-axle tractors in agricultural enterprises of legal entities of 5,877, almost half, 2,469, are under ten years old, and in the Vojvodina region, of 431 tractors, 116 are under ten years old (Radivojević et al. 2014). In 2010, the total number of two-axle tractors in Montenegro was 4,560, of which 123 were owned by economic entities (companies, cooperatives, cooperatives, etc.), with 55.6% of tractors older than 25 years and 38.16% younger than 5 years (Koprivica et al., 2020). Poje (2016) states that in Croatia 91.8% of registered tractors are older than 10 years. The average age of registered tractors is 28.44 years, and 20.71 years in Osijek Baranja County (Zimmer, 2019). In Slovenia, of the total number of registered tractors in 2014, 83 291 were older than 12 years. The average age of registered tractors is over 21 years (Poje, 2016). In Hungary, the average age of tractors was 18.3 years in 2013 and about 12 years in 2016. Of all categories of tractors, 57% are older than 10 years. The tractors that are younger than 10 years are mostly tractors with a power of more than 60 kW (Kesmarki-Gally et al., 2017). In Turkey, of the total number of tractors, 46% are older than 25 years, while in the western part of Turkey, 88% of tractors are younger than 20 years old (Özpinar, 2020) .



Graph. 1. Representation of two-axle tractors in the cooperative by category in relation to total engine power

For agricultural production in Serbia, in addition to 597,816 tractors, over 31,000 different combines and 2,421,065 implements are used. There are 832,000 mechanization devices for soil cultivation, over 122,000 mechanization devices for fertilization with manure and mineral fertilizers, about 130,000 different sowing and planting machines, about 220,000 mechanization devices for plant care and protection, as well as 300.000 trailers and over

287,000 mechanization units for the use of forage crops, 75,000 technical means for irrigation, and about 410,000 different means for silage collection, mechanical milking, storage, processing, and packaging (Božić et al., 2014). In the production of agricultural crops, all machines used to perform agrotechnical work are divided into six groups, namely machines for: Soil preparation, sowing and planting, plant care, plant protection, harvesting and transport (Tab. 2).

In total, 76 different connection machines from all the above groups are used in the cooperative. The most tillage machines are 42.10% or 32 units, of which the most are plows (12), then heavy disk harrows with packer rollers (7), combine harvesters (5) and an equal number of rippers and harrows, 4 units each. In the tillage machinery group, ranked by number, there are 12 tillage machines or 15.79% of the total number of machines, including 8 inter-row cultivators and 4 mineral fertilizer spreaders. Seed drills account for 11.84% of the total number of tillage machines, including 8 seed drills for hoe seeding (wide row seeding) and one seed drill for foreign grain (short row seeding). The number of 8 crop protection machines (sprayer) and transport (trailer) is the same, which accounts for 10.53% of the total number of cultivated machines. In addition to tractor trailers, the cooperative also uses 7 trucks with 9 trailers for transportation. The group of machines used for harvesting arable crops includes 2 implements each (rotary mower, rake and beet cleaner) and a front loader (Tab. 2). Not included are 8 self-propelled universal combines for harvesting small cereals, soybeans and corn, and 14 self-propelled combines for sugar beets.

Table 2. Number of connecting machines by agrotechnical measures - by farm groups

Name of the machine group	Number of units (pieces)	Representation (%)
I Soil preparation machines	32	42,10
II Seeding and planting machines	9	11.84
III Plant care machinery	12	15.79
IV Machines for plant protection	8	10.53
V Machinery for transport	8	10.53
VI Harvesting machinery	7	9.21
Total	76	100

In the cooperative, one cultivating machine should work an area of 34.11 ha, and one tractor requires 2.23 machines (Table 3). In Serbia, on average, a farm has 3.12 resources for the implementation of agrotechnical measures from the above six working groups. According to statistical data, 94% of connected machines are older than 10 years (Božić et al., 2014). The analysis of the cooperative's energetic equipment concludes that it has 35 tractors with a total engine power of 3,655.0 kW and an average power of 104.43 kW. The given value is lower than the average tractor power of 120.3 kW in the farms of legal entities and entrepreneurs (cooperatives) in Serbia (Božić, 2014) and higher than the average tractor engine power in the USA, Australia and Canada (62.5 kW), in Western Europe (45.3 kW) and in Russia (65.1 kW) (Pawlak et al., 2002).

Table 3. Energy equipment of the cooperative

Parameters	Unit of measurement	Value
Total arable land	ha	2,592
Total number of tractors	com	35
Total power of tractors	kW	3,655.0
Average tractor power	kW	104.43
Energy equipment of the tractor	kW/ha	1.41
Specific load of the tractor with arable land	ha/kW	0.71
Number of units per tractor area	ha/ tractor	76.23
Number of tractors per 100 ha	com	1.31=0.0131
Total number of connected machines	com	76
Number of implements per tractor	com	2.23
Number of arable land per connected machine	ha/machine	34,10

In Radić study of six cooperatives in Vojvodina (2016), energy endowments ranged from 1.13 to 1.95 kW/ha, with an average of 1.40 kW/ha, which is consistent with the study in this paper of 1.41 kW/ha. The use of mechanical tractor power in America is 0.783 kW/ha, in Europe 0.694 kW/ha, in Germany 1.7 kW, in Montenegro 1.18 kW/ha (Poje, 2016; Koprivica et al., 2020). Radić (2016) states that the average specific load of tractors on arable land in the Republic of Serbia is 0.911 ha/kW and in the studied cooperatives 0.77 ha/kW (0.51-1.04 ha/kW), which is consistent with the research results of the cooperative "Agroprom" (0.71 ha/kW). The results of Pawlak et al. (2011) show that the energy availability is 0.351 ha/kW in the USA, Australia and Canada, 0.147 ha/kW in Western Europe and 0.651 ha/kW in Russia, which is less than the results presented in this paper. According to the results of Radivojević et al. (2014), farms of legal entities and entrepreneurs in the Republic of Serbia cultivate 93.6 ha/tractor of agricultural land with a two-axle tractor, which is more than the results presented in this paper of 76.23 ha/tractor. Table 3 shows that there are 1.31 tractors per 100 ha in the cooperative, which is higher than the Serbian average of 1.11 tractors for farms of legal entities and entrepreneurs (Nikolić et al., 2013). In the USA, Australia and Canada there are 2.05 tractors per 100 ha of arable land, in Western Europe 8.92 and in Russia 0.70 (Pawlak et al., 2002).

Conclusions .

The equipment of the agricultural cooperative "Agroprom" from Stara Pazova with modern agricultural machinery is at a satisfactory level, because with 35 two-axle tractors and 79 attachments it cultivates 340 ha of land owned by . In addition, the cooperative provides services to other agricultural producers and subcontractors with its machines on an area of 2,252 ha, generating a significant source of income. The total available rated power of all tractors is 3,655.0 kW. Most of these are John Deere tractors (15), so they account for more than half (51.78%) of the total rated tractor power at 1,877.78 kW. A two-axle tractor with an average engine power of 104.43 kW works 76.23 ha with 2.32 implements. There are 1.31 tractors per 100 ha of arable land. The power equipment of tractors in the cooperative is 1.41 kW/ha or 0.71 ha/kW. The average age of tractors is less than 10 years, with the youngest tractors being 5 years old (John Deere 6530) and the oldest 12 years old (MTZ-1025).

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Application of the insecticide tefluthrin for the control of Elateridae and Scarabaeidae in maize crops

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Abstract

Achieving high and stable yields with high nutritional value, that are economically profitable and at the same time safe for health is the primary goal of maize production. Soil pests (Elateridae and Scarabaeidae) are among the economically important pests of maize. To control these pests, field trials were conducted in 2021 in the region of Vojvodina province in the localities of Kovilj and Čurug on mercantile maize crops (hybrid NS 3022 (FAO group 360)), according to standard EPPO methods. Products based on the active ingredient tefluthrin (5 g a.i./kg, GR) at the rate of 15 kg/ha and (15 g a.i./kg, GR) at the rate of 7 and 10 kg/ha, were applied simultaneously with sowing with depositors. The experiment was laid out in four replications. Immediately before sowing, soil analyzes were carried out to determine the number of pests. In the first evaluation, the effects of the applied insecticides were derived from the number of sprouted plants at a distance of 10 m and the number of plants damaged by Elateridae and Scarabaeidae larvae. In the second assessment, the mass (g) and height (cm) of 25 plants per replication were evaluated. Results are presented as average values and significance of differences (LSD 5%) at ANOVA. Before sowing, at the locality Kovilj only Elateridae larvae (2.1/m^2) were determined, while at the locality Čurug larvae of Elateridae (1.8/m^2) and Scarabaeidae (1.3/m^2), were found. The analyzed tefluthrin based products significantly increased the number of sprouted plants, decreased the number of damaged plants and increased the mass and height of maize plants. The results indicate that populations of Elateridae and Scarabaeidae are sensitive to the insecticide tefluthrin.

Key words: maize, Elateridae, Scarabaeidae, tefluthrin, efficacy

Introduction

A significant number of pests appear on maize, leading to both quantitative and qualitative yield losses. Of the total 130 harmful species occurring on maize in the Republic of Serbia, insects account for about 70%, of which beetles and moths are the most important (Čamprag et al., 2002). Soil pests (fam. Elateridae and Scarabaeidae) belong to one of the most economically important pests of maize. Click beetles (fam. Elateridae) are distributed worldwide and are among the most dangerous pests of crops. They cause the greatest damage in grain-growing regions, especially in Vojvodina. Several genera and species are known, of which *Agriotes* is the most widespread and economically important genus, living in the larval stage, on chernozem and meadow black soil. The larvae of the *Agriotes* genus are extremely phytophagous and are regular pests of crops, while the larvae of other genera and species are less harmful and often appear as saprophages, necrophages or predators. Therefore, for proper protection, it is very important to know the faunal composition of wireworm larvae in each plot (Kereši et al., 2018). The harmfulness of wireworms is particularly pronounced during a warm and dry spring. During feeding, they ingest only the liquid fraction, so they are forced to grind and crush large amounts of plant tissue. They damage the root or the root neck of the plants, whereupon they wither, turn yellow and die in an oasis-like manner, as the horizontal spread is characteristic of these pests. This causes an incompleteness of plant structure and the need for additional plant seeding (Kereši et al., 2018). Cockchafers (family Scarabaeidae), both as adults and larvae, feed on plants, carrion, etc. The adult insects of some species feed on the leaves of fruit trees, and when they occur in masses, they can bare the trees, but, the main damaging stage is the larvae (Kereši et al., 2018). The larvae are scarab-shaped grubs, that are polyphagous, live in the soil and feed on the roots of various plants. This family includes numerous species (over 30,000), divided into several subfamilies, of which the most important for agriculture are Melolonthinae, Rutelinae and Cetoniinae (Kereši et al., 2018).

Application of plant protection products (PPPs) and suitable agrotechnical measures are indispensable measures in the modern production of maize or any other agricultural crop. To achieve high and stable maize yields, one of the prerequisites is well-planned pest control. The widespread use of insecticides in agriculture and their increasingly intensive use to increase the yield of agricultural products and achieve the best possible results can lead to undesirable effects on crops and the environment. Seed treatment of maize as a protection measure is widely accepted. In the Republic of Serbia, PPPs based on tefluthrin and cyantraniliprole are approved for this purpose (Petrović and Sekulić, 2021). In addition to insecticides used to treat maize seeds to protect seedlings, soil insecticides are also used. Previously, there were several products with different active ingredients on the market, but after many products were banned, only the tefluthrin-based PPP remained. The active ingredient tefluthrin binds well to the soil and to the surface of plant roots, so it also works well against pests that feed on roots or underground plant parts. Also, tefluthrin goes into a gas phase in the soil and has a repellent effect, i.e. it repels soil pests around the seeds.The aim of the research in this paper is to improve the protection of maize crops from soil pests, along with continuous monitoring of the occurrence of soil pests in maize crops and the evaluation of the effectiveness of the tested plant protection products based on tefluthrin.

Materials and Methods

The trial was designed according to standard OEPP/EPPO methods for experimental design and data analysis (Anonymous, 2012), for phytotoxicity (Anonymous, 2014), and for evaluating the efficacy of insecticides in controlling soil pests (larvae of the fam. Elateridae and Scarabaeidae) (Anonymous, 2004). The experiments were carried out in 2021 at the localities of Kovilj and Čurug (Vojvodina, Serbia). Before conducting the experiment, a soil analysis was performed to determine the presence and number of soil pests (by digging pits with a depth of about 45 cm and a size of 25×25 cm, sifting and examining the soil and determining the number of larvae/m^2). Soil analysis for the presence and abundance of soil pests was carried out in the accredited *Laboratory for Biological Research and Pesticides, Department of Plant and Environmental Protection, Faculty of Agriculture in Novi Sad*. The experiment was set up in a completely randomized block system in four replicates for each product, as well as the untreated control. Plant protection products based on tefluthrin (5 g a.i./kg, GR) were applied at the rate of 15 kg/ha, and tefluthrin (15 g a.i./kg, GR), was applied in the amount of 7 and 10 kg/ha simultaneously with sowing, depositor. The experiments were carried out on fresh, mercantile maize, hybrid NS3022 (FAO group 360). Sowing in the field was done by machine, with 70×25 cm spacing. During the trial, two evaluations of the effects of the tested plant protection products were performed, namely:

- I assessment after emergence - number of sprouted plants per 10 m and the number of damaged plants;
- II assessment at the stage of 5-6 leaves - mass (g) of green plants and height of the aerial part (cm) 25 plants per replicate.

The obtained data are expressed by the average values ($\bar{x}$) for the number of sprouted plants, the number of plants damaged by Elateridae and Scarabaeidae larvae, the mass and the height of the aerial part of the plants. Then, the deviations from the mean values (standard deviation, Sd±) and the significance of the differences for the analyzed varieties were determined for the 95% confidence level, relative values compared to the control (K=100%), and the percentage of damaged plants in relation to the total sprouted plants (%).

Results and Discussion

The results of the efficacy activity of PPPs based on tefluthrin in control of larvae Elateridae and Scarabaeidae families in maize crop at the localities of Kovilj and Čurug are shown in Tables 1-2. Before conducting the experiment, soil analysis was carried out, and the number of larvae of the fam. Elateridae was determined. At the locality of Kovilj it was 2.1/m^2 and in Čurug 1.8/m^2, while the number of larvae of fam. Scarabaeidae at the Čurug site was 1.3/m^2.

Locality Kovilj. The average number of sprouted maize plants per 10 m length ranged from 27.9 to 36.9 for the varieties where insecticides were applied. Regardless of the applied amount, the tested insecticides caused a significant increase in the number of plants from 19.4 to 32.2% compared to the control (Table 1). A significant reduction in the number of plants damaged by larvae of the Elateridae family compared to the control was observed when applying the tested plant protection products (tefluthrin 5 g a.i./kg and tefluthrin 15 g a.i./kg, GR), with the average number of damaged plants ranging from 8.5 in the control to 1.5 in the variants where insecticides were applied. The tested products provided a significant reduction in the number of damaged plants compared to the control (Table 1). The mass of green maize plants when the insecticides were applied was a significantly higher compared to the control (Table 2). The tested insecticides ensured 28.4-51.7% higher mass of plants compared to the control. The height of the maize stem at the stage of 5-6 leaf stage varied on average from 68.7 cm in the control to 81.4 cm in the variants where insecticides were applied. Products based on tefluthrin provided significantly higher maize stem height compared to the control (14.6-18.5% higher).

Locality Čurug. The average number of sprouted maize plants per 10 m length ranged from 27.5 to 33.6 in the variants where products based on tefluthrin were applied. Regardless of the amount applied, the insecticides resulted in a significant increase in plant number from 18.2 to 21.5% compared to the control (Table 1). A significant reduction in the number of plants damaged by the larvae of fam. Elateridae and Scarabaeidae was observed when tefluthrin was applied compared to the control, with the average number of damaged plants ranged from 7.5 in the control to 2.0 in the variants where insecticides were applied. Tefluthrin caused a significant reduction in the number of damaged plants by 5.9-9.2% compared to the control (Table 1).

Table 1. Average number of sprouted plants at distance of 10 m and the number of damaged plants from Elateridae and Scarabaeidae larvae (Kovilj and Čurug, 2021)

Insecticide (kg/ha)	Average number of sprouted plants at distance of 10 m	K 100%	The number of damaged plants	K 100%
	$\bar{x}$ ±Sd		$\bar{x}$ ±Sd	
	Locality Kovilj			
tefluthrin 5 g/kg GR (15)	**34.3**±1.5 a	**122.9**	**2.50**±1.0 b	**7.3**
tefluthrin 15 g/kg GR (7)	**33.3**±2.4 a	**119.4**	**2.75**±0.9 b	**8.3**
tefluthrin 15 g/kg GR (10)	**36.9**±1.9 a	**132.2**	**1.50**±0.6 b	**4.1**
control	**27.9**±4.8 b	/	**8.50**±2.1a	**30.5**
LSD 5%	3.95		1.76	
F, p - values	F=6.57; p<0.01		F=1.68; p<0.01	
	Locality Čurug			
tefluthrin 5 g/kg GR (15)	**32.6**±1.3 a	**118.5**	**3.00**±0.8 b	**9.2**
tefluthrin 15 g/kg GR (7)	**32.5**±1.9 a	**118.2**	**2.75**±0.9 b	**8.5**
tefluthrin 15 g/kg GR (10)	**33.4**±1.5 a	**121.5**	**2.00**±0.8 b	**5.9**
Control	**27.5±**2.7 b	/	**7.50**±2.1a	**27.3**
LSD 5%	2.54		1.70	
F, p - values	F=7.82; p<0.01		F=2.46; p<0.01	

$\bar{x}$– average value; Sd± - standard deviation; K 100% - relative value compared to the control

The mass of green maize plants is significantly higher when tested PPPs are applied compared to the control (Table 2). Tefluthrin ensured 28.5-34.9% higher plant mass compared to the control. Height of the maize stem ranged on average from 52.0 cm in the control to 67.3 cm in the tested varieties. Products based on tefluthrin provided 19.6-29.4% higher maize stem height compared to the control. Application of the product with tefluthrin (5 g a.i./kg GR) in the amount of 15 kg/ha and tefluthrin 15 g a.i./kg GR) in the amount of 7 and 10 kg/ha in the localities of Kovilj and Čurug did not cause reduction of germination, and no other changes in the form of deformation or changes in the color and shape of leaves on maize, hybrid NS3022 were registered.

Based on available literature sources, there are no data on resistance of soil pests to the insecticide tefluthrin. However, in insect populations that are intensively controlled with this active ingredient or others from the group of pyrethroids, a change in sensitivity is possible, and therefore the instructions of the anti-resistance strategy should be followed (IRAC, 2021).

Table 2. The mass of 25 plants and the average plant height (cm) (Kovilj and Čurug, 2021)

Insecticide (kg/ha)	Mass (g) maize plants $\bar{x}$±Sd	K 100%	Average height (cm) plant $\bar{x}$±Sd	K 100%
	Locality Kovilj			
tefluthrin 5 g/kg GR (15)	**2950.0**±581.6a	**151.1**	**78.7**±5.9 a	**114.6**
tefluthrin 15 g/kg GR (7)	**2507.5**±360.2a	**128.4**	**79.3**±4.1 a	**115.4**
tefluthrin 15 g/kg GR (10)	**2962.5**±167.8a	**151.7**	**81.4**±5.6 a	**118.5**
control	**1952.5**±183.0 b	/	**68.7**±5.6 b	/
LSD 5%	593.5		8.05	
F, p - values	F=6.18; p<0.05		F=4.50; p<0.01	
	Locality Čurug			
tefluthrin 5 g/kg GR (15)	**1300.0**±108.6a	**128.5**	**67.3**±7.2 a	**129.4**
tefluthrin 15 g/kg GR (7)	**1312.5**±110.8a	**129.8**	**62.2**±3.8 a	**119.6**
tefluthrin 15 g/kg GR (10)	**1365.0**±77.20a	**134.9**	**64.3**±4.2 a	**123.7**
Control	**1011.3**±134.9 b	/	**52.0**±2.6 b	/
LSD 5%	144.42		6.28	
F, p - values	F=8.46; p<0.05		F=2.69; p<0.01	

$\bar{x}$– average value; Sd± - standard deviation; K 100% - relative value compared to the control

Based on research conducted by Van Herk and Vernon (2007), wireworms exposed to seed pretreated with tefluthrin after 15 minutes (and less) of contact with the seed were reppeled. According to the same author (Van Herk et al., 2008), larvae of *Agriotes obscurus* and *Limonius canus* exposed to wheat seeds treated with products based on tefluthrin, were repelled from the seeds (after less than 20 minutes), except in the case of larval <50%. Hall (2003) reported the efficacy of a tefluthrin-based product in controlling *Melanotus communis* (Gillenhal). The tested insecticide significantly reduced damage to maize plants by 70% compared to the untreated control. The study by Cizej et al. (2017) showed statistically significant differences in the comparison between the control variant (untreated soil) and the soil treated with tefluthrin, with a high efficiency in repelling *Agriotes* sp. observed in the maize crop. A significant decrease in the number of maize plants per 10 m^2 and in their average height was also observed in the control variant, which is consistent with this study.

Conclusion

At the localities Kovilj and Čurug, the tested products based on tefluthrin (5 g a.i./kg and 15 g a.i./kg, GR) ensured a significant increase in the number of plants, a significant decrease in the number of damaged plants compared to the control, and a greater mass and height of the maize stem in the initial stages of growth compared to the control. This shows that the larval populations of the families Elateridae and Scarabaeidae are sensitive to tefluthrin in the mentioned localities, which gives the possibility to continue using this insecticide following the recommendations of the anti-resistance strategy.

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First testing of horse-drawn roller-cutter for green-manure crops management in Croatia

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Abstract

Green manuring (GM) for raising the soil fertility becomes increasingly important due to environmental and other sustainability issues. However, GM is unavoidably associated with additional agrotechnical operations and energy consumption required for establishment of GM crops and their termination or incorporation of a GM herbage into soil, depending on the soil tillage system. Horse powered agriculture could be an option to decrease or avoid the consumption of fossil energy, as well as CO_2 emissions from powering the cultivation of arable crops. However, some operations in GM management might be challanging by using solely the living horse power, namely the chopping of GM's hergabe before its incorporation into soil. An entirely CAD-designed retroinnovation developed in Schaff mat Paerd asbl. of Luxembourg has given a horse-drawn alternative to tractor powered choppers and mulchers. The horse-drawn roller-cutter showed a good performance on crimson clover (*Trifolium incarnatum* L.) GM crop at the experimental site near Požega. However, the broad acceptance of horse-powered farming might be chalanging due to decrease of human labor efficiency when compared to modern tractorized farming. Rising the consciousness about sustainability issues and need for improved resilience to changing climate, as well as personal inner transformation of people might be needed to improve the image of such a labor-intensive agriculture.

Key words: green manuring, fossil energy consumption, sustainability, CO_2 emissions, low-

input farming, inner transformation

Introduction

Green manuring (GM) for raising the soil fertility becomes increasingly important due to environmental and other sustainability issues. Namely, predominant reliance on the synthetic nitrogen (N) mineral fertilizers for achieving the high crop yields since the Green revolution has been associated with many detrimental consequences. According to Basosi et al. (2014), mineral nitrogen fertilization represents one of the main agricultural practices with a high emission of pollutants into the atmosphere, soil and water. De Vries (2021) indicates the need to reduce the N emissions, both from agriculture and from traffic and industrial sources. Moreover, in the times of prospective depletion of fossil fuels that are necessary for powering the synthesis of mineral nitrogen fetilizers, and climate policies that push the industries and societies to minimize the CO_2 and N_2O emissions, and to decrease the reliance on fossil energy resources, green manuring may become one of the core options to maintain or rise the soil fertility and crop yields. However, GM is unavoidably associated with additional agrotechnical operations and energy consumption required for establishment of GM crops and their termination or incorporation of a GM herbage into soil, depending on the soil tillage system (Ambrušec et al., 2021; Wu et al., 2011). Inspired by the need to reduce the consumption of fossil fuels, Ambrušec et al. (2021) have proposed the use of animal work for powering the agrotechnical operations by smallholdings and small-scale farms. This is in line with the principles of low-input farming (Parr et al., 1990; cit. Poux, 2007) where should be optimized the farmers' reliance on the on-farm produced inputs, while closing the cycles of nutrients and energy, and minimizing the adverse environmental impacts.

According to the author's previous experience, in agronomy where the main crop is seeded into a conventionally prepared seedbed (i.e. into a fine structured bare ground), green manuring can be challenging if there is present a huge and tall GM herbage on the soil surface. The problem rises when there are used small plows, like horse drawn plows, that can hardly completely cover the tall herbage with the soil due to the shallow plowing depth. In a large-scale arable farming there are being used tractor-powered choppers or mulchers with working width of 2 to 3 m and great efficiency. In small-scale farming and small-plot fields there are often being used small mulchers. In the previous experiments of Ambrušec et al. (2021), the GM herbage was chopped by a small gasoline-powered mulcher (Honda F-620 Y + MIO Standard Osijek mulcher) with aim to ease the plowing herbage under, to speed up the herbage decomposition, and release of plant nutrients from herbage into soil (Photo 1). However, if there is an ambition to completely avoid the consumption of fossil fuels, this requires an alternative implement to chop the herbage before plowing it under. Recently, the Schaff mat Paerd asbl. of Luxembourg has designed a prototype of roller-cutter aimed for chopping the herbage mass for green manuring (photos 2 and 3). This roller-cutter is intended for small farms that employ animal power for agricultural operations. Therefore, it is a light-weight implement with cutting blades driven by rolling action of oval knive-fitted stainless steel rings around a rubber roller, rolled over the soil surface. Aim of this preliminary research was to obtain a quick appraisal of the roller-cutter's performance in chopping the green manure's herbage for subsequent plowing it under and seedbed preparation.

Photo 1. Gasoline-powered mini-tractor Honda F-620 Y coupled with MIO Standard mulcher used to chopp the crimson clover herbage in previous researches

Photo 2. Blades on steel rings over the rubber drum on horse-powered roller-cutter for chopping the crimson clover herbage in this research (designed by Schaff mat Paerd asbl., Luxembourg)

Photo 3. Double traction shafts in front of the roller-cutter for hitching a single horse

Material and Methods

The investigated implement was the SmP Kombi-Roll Concept 3.0, the 3rd generation roller-cutter of the Schaff mat Paerd asbl. of Luxembourg. It consists of an inner rubber roller vulcanized on a steel drum that carries 5 stainless-steel floating rings that copy the terrain and bear 12 stainless-steel chopping blades on each ring (Figure 1). The inner roller is coupled to a frame by two greasable ball bearings. Frame is equipped with two pneumatic wheels for the transport and a screw handle for lifting the roller together with the frame. Frame is coupled with a double traction shafts adapted to hitch a single horse. Total weight of the implement is 200 kg.

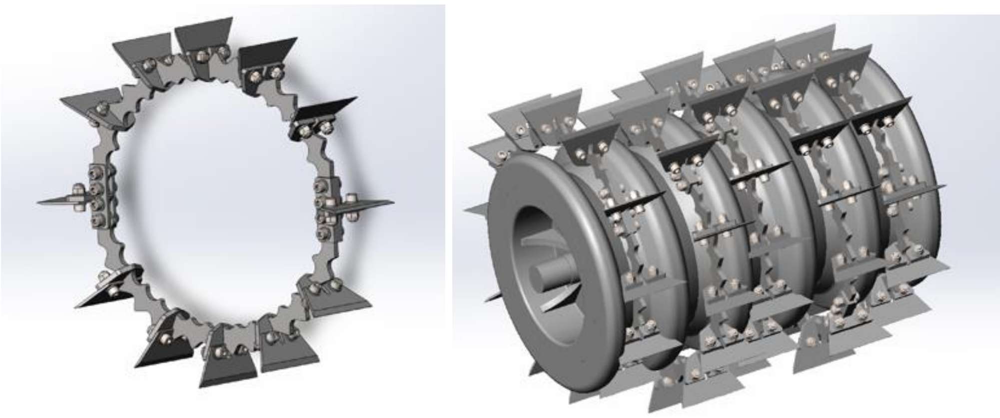

Figure 1: floating stainless-steel ring (left), and rings mounted on rubber roller (right)

Testing was performed on a small field near Požega town (arable parcel of 3000 m^2 in total, but rolling was done on less than a half of the area), on 22nd April 2023, on a crimson clover (*Trifolium incarnatum* L.) crop grown for green manuring, with some volunteering oats.

Crimson clover was seeded in early September 2022. At places where the chop length was measured, before the pass of the implement the crimson clover plant height was between 38 and 43 cm (Photo 4), and the clover's stand and canopy were dense. Crimson clover was in a late vegetative stage with soft stems. The roller-cutter was drawn by the Croatian Heavy Draft Horse mare (Photo 5), approximate bodyweight of 650 kg.

Photo 4. Intact herbage mass of the crimson clover before chopping

Photo 5. Croatian Heavy Draft Horse mare with the roller-cutter hitched

Results and Discussion

Testing has proven that the crimson clover can be efficiently laid down and chopped in just a single pass with the horse-drawn roller-cutter, with no use of fossil fuel. The investigated roller-cutter obviously needed just a little of energy for its operation. Namely, the mare which pulled the implement didn't show signs of fatigue during the work, thus indicating that there were low traction forces required. Effectivity of the roller-cutter was quite good. All the present herbage of crimson clover was cut and laid down, with no intact plants remained, thus enabling for easy plowing-under the herbage. Chop lengths after a single pass of the implement were from 17 to 37 cm (Photo 6).

Photo 6. Chopped crimson clover herbage mass after a single pass of the roller-cutter

Working width was 60 cm, working speed about 4,5 km/h and time required for a turn was about 10 s. Under the assumption that small farmers' plots in the north-eastern Croatia have a length of 400 m, there would be theoretically required about 0,09 hours or 5.4 minutes for a single pass. With the anticipated rest of a horse and man, the time required for a single pass would be about 6 minutes. The laid and chopped area of a single pass would be equal to 240 m^2 or 0,024 ha per pass. Under the assumptions that the time required for harnessing, hitching and arrival to a field would be half on an hour, and the same for the return back home, then in the 8-hours working day there would remain 7 hours available for the field work. Under the assumption that a team of single horse and man can work 7 hours per day in a field, the work efficiency might be up to 70 passes 400 m long. i.e. 16800 m^2 or 1.68 ha per working day.

The above presented working efficiency is theoretical and therefore should be tested in practical farming where the eventual fatigue of horse and man could appear due to greater working area than in this preliminary trial.

Although the draft force was obviously low, for proving this there would be required measurements by field dynamometer (Schmit et al., 2022). In order to achieve a high-quality appraisal of the tested implement, there would be required to continue the testing on various GM herbages differing in maturitiy, plant species, plant height and stem rigidity.

Despite the proven feasibility of horse-drawn agriculture to cope with challanges associated with green manuring (at least with soft-stemmed crimson clover), the horse traction may not face the broad acceptance by the agricultural experts and general public, mainly due to very low efficiency of human labor. Namely, a single tractor-operater and 75 kW tractor equipped with 3.2 m working width mulcher can do 1.2 ha/h, or 9.6 hectares in one working shift (8 hours), what is 5.7 times more than the projection for the horse-drawn variant. Modern people could dislike the dramatical decrease of human labor efficiency, or the related increase of the need for human labor in the horse-drawn agriculture, not only because of lower efficiency, but also due to it is less convenient than sitting in a tractor cabin or doing office-based jobs and jobs associated with soft skills. Rising the consciousness of the sustainability issues and need for climatic resilience, as well as the personal inner transformation of people might be needed to make the horse-drawn agriculture appears more attractive. The greater need for human labor in agiculture might induce the return of people to rural areas and countryside thus relieving the cities and lessening the urban waste generation. Namely, urban waste generation per capita is always greater than of rural people (Gómez-Sanabria et al., 2022), and rural waste is more easily recycled or reused (Bernardes and Günther, 2014).

Conclusion

Newly designed roller-cutter SmP Kombi-Roll Concept 3.0 has shown an excellent performance in laying down and chopping the crimson clover herbage in a late vegetative stage when intended for green manuring. Working speed was about 4.5 km/h and working width 0.60 m, thus enabling the theoreticaly projected work efficiency of 1.68 ha per 8-hours working day, with included short rests for man and horse, and 1 hour for hitching, getting to a field, getting back and hitching-off. The projected work efficiency is much lesser than of a modern 75 kW (100 hp) tractor equipped with 3,2 m working width mulcher, that can do 1,2 ha/h, or 9,6 hectares in one working shift (8 hours), which is 5.7 times more than the projection for the horse-drawn variant. Due to much lesser effectiveness of human labor and poorer convenience of a worker, there is not anticipated a broad acceptance of horse-drawn technology nowadays, except in small organic farms and marginal areas of agriculture, like in mountains. However, consciousness about sustainability issues, and need for climate resilience might favour the acceptance even in major agricultural regions. For a better acceptance there is needed education of stakeholders in food chain, as well as the personal inner transformation (Woiwode et al., 2021) of people and farmers.

Acknowledgements

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Prvo testiranje valjka-sjekača na konjsku vuču za zelenu gnojidbu u Hrvatskoj

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Sažetak

Zelena gnojidba (ZG) za podizanje plodnosti tla postaje sve važnija zbog ekoloških i drugih pitanja održivosti. Kakogod, ZG je neizbježno povezana s dodatnim agrotehničkim zahvatima i utroškom energije potrebnim za uzgoj ZG usjeva i njihovu terminaciju ili inkorporaciju u tlo, ovisno o sustavu obrade tla. Poljoprivreda na konjski pogon mogla bi biti opcija za smanjenje ili izbjegavanje potrošnje fosilne energije. Međutim, neke radnje u provođenju zelene gnojidbe mogu biti teže izvedive korištenjem samo žive konjske snage. Naime, usitnjavanje zelene mase biljaka prije unošenja u tlo sada se radi traktorskim malčerima, a alternativa bez utroška goriva do sada nije bila poznata. Retroinovacija napravljena u Schaff mat Paerd asbl. iz Luksemburga dala je alternativu traktorskim sjeckalicama i malčerima. Alternativni valjak-sjekač na konjsku vuču pokazao je dobre rezultate na usjevu inkarnatske djeteline na pokusnom mjestu kod Požege. Ipak, prihvaćanje poljoprivrede na konjski pogon može biti problematično zbog smanjenja učinkovitosti ljudskog rada u usporedbi s modernom traktoriziranom poljoprivredom. Podizanje svijesti o pitanjima održivosti i osobna unutarnja transformacija ljudi možda će biti potrebna kako bi se poboljšala slika takve radno intenzivne poljoprivrede.

Ključne riječi: sideracija, potrošnja fosilnih goriva, održivost, emisija CO_2, poljoprivreda s malim ulaganjima, unutrašnja transformacija

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The application of advanced technologies in the research of *terroir* factors in viticulture and oenology

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Abstract

Faced with a very picky market and strong competition from high-qulity imported wines, it is necessary to shed light on the *terroir* characteristics of local wine-growing areas and the typicality of local wines, as this represents an opportunity to enhance the wine sector through geographical indications and the protection of small appellations. A necessary step in this complex process is the application of advanced technologies, i.e. new techniques and different modelling methods, accompanied by a spatial analysis of different parameters through the geographic information system (GIS).

This paper presents several examples of application of advanced technologies and development of innovative modelling methods in viticulture and winemaking in the Oplenac wine-growing district (Serbia), all of which have the potential for wider application and adaptation of developed methods to conditions in other wine-growing areas of the region. With the aim of studying the *terroir* factors, this paper presents modelling performed by applying the Analytical Hierarchy Process (AHP) method, which was used for modelling and classification of the studied abiotic *terroir* factors in hierarchical levels. The modelling and classification of the abiotic *terroir* factors in hierarchical levels was performed using the Geographical Detector Method (GDM). Modelling with TOPSIS method was used in this work to present an example of modelling and ranking of analysed anthropogenic *terroir* factors. The application of the GIS technology was used in this paper to present examples of mapping favorability classes of abiotic and anthropogenic *terroir* factors and small appellations in particular absolute, elite, historic and organic vineyards, i.e. viticultural parcels. The Random Forest Clustering (RFC) method was used to present examples of wine quality and typicality prediction. Through this modelling, a link is established between the *terroir* factors studied and wine quality and typicity, which forms the basis for the protection of geographical indications in the EU PDO/PGI system. Finally, a comprehensive classification of viticultural micro-areas was made based on all the viticultural-oenological models developed.

Key words: abiotic and anthropogenic *terroir* factors, modelling, multicriteria analysis, GIS

Introduction

Research on *terroir* factors occupies a central place in the European Union (EU) PDO/PGI system, as legislation requires evidence of a significant or decisive effect of specific natural and anthropogenic factors existing in the wine-growing area in question on the quality and characteristics of wine from that area (for PDOs). Determining causal relationships between the characteristics of a wine-growing area and the quality and characteristics of the wine, especially in the case of PDO designations, requires detailed research and extensive knowledge of the *terroir* of the area in question (Ninkov *et al.*, 2019), which requires extensive scientific research and expert analysis. Exploring the multifactoriality of *terroir* requires significant effort, data, and scientific knowledge. However, certain advanced technologies are currently being used for these purposes, some of which have been identified in the OIV Zoning Resolution (Resolution OIV-VITI 423-2012) and the Precision Viticulture Resolution (Resolution OIV-VITI 593-2019).

Numerous *terroir* research activities in the Western Balkans region were carried out in Serbia, where, in addition to zoning of wine-growing areas, small appellations have been introduced, which are actually traditional terms for wines with geographical indications: absolute, elite and historic viticulture parcels, as well as organic viticulture parcels (organic wine-growing locations and micro-areas). For the use of the names of the small appellations on the labels, i.e. for the use of the corresponding traditional terms, it is necessary that the micro-area in question has extremely favourable or ideal soil, climatic, topographic and other necessary conditions for the successful cultivation of vines and the production of quality wines with the geographical indications, which is confirmed by the results obtained through the application of advanced technologies and corresponding innovative models.

For the research of *terroir* factors and their modelling, in addition to the application of the geographic information system (GIS), remote sensing, GPS devices and different software solutions, different models were used in this paper for the multicriteria analysis. One of the most important of the models used is the Analytical Hierarchy Process (AHP) method. This is a multi-attribute decision making (MADM) method that provides a powerful logical approach to solving complex multi-criteria decision types, breaking down the complex problem to obtain a hierarchical structure that shows the relationships among the objective, criteria, sub-criteria, and alternatives (Saaty, 1980; 2012). The Geographical Detector Method (MGD) applied in this paper relied on the use of the Geodetector programme, a software for measuring and assigning stratified (layered) heterogeneity. Stratified heterogeneity refers to phenomena that show more similarity within strata than between strata (http://www.geodetector.cn/). An important model for multicriteria analysis is the Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) method. It is a ranking method proposed by Hwang and Yoon (1981) for solving multicriteria decision problems (Srđević, 2002). Random Forest Clustering (RFC) method is a machine learning method that uses a large number of nonlinear algorithms and a statistical learning method designed for classification, regression, and other tasks solved by constructing multiple decision trees and finding a class (classification) or a median/average prediction (regression) of the individual trees (Ho, 1995; 1998).

By applying these methods, i.e., machine learning with spatial analysis, many complex questions related to the study of *terroir* factors in viticulture and oenology can be answered.

Material and Methods

The realisation of the innovative modelling in this paper was carried out through research activities aimed at the multifactorial *terroir* potential, i.e. through: terrain research with application of the GIS technology and GPS devices; laboratory analysis of soil and wine; preparation and statistical and geostatistical data processing; modelling and mapping with application of appropriate methods, GIS, software and extensions; as well as through validation of the obtained results and the obtained innovative models. Examples of viticultural-oenological modelling are presented for the area of the Oplenac wine-growing district

(Sumadija wine-growing region).

Research of location, i.e. spatial data of 836 commercial vineyards in the area of Oplenac wine-growing district was carried out by two different applications of GIS technology: application of only geospatial technology and relevant spatial maps and application of GPS devices and geospatial technology. Spatial exploration of vineyards was carried out according to the instructions of the Joint Research Centre of the European Commission for surveying vineyard parcels in the system for their control (Kerdiles *et al.*, 2008).

For the spatial exploration of existing and former vineyards, as well as for the general exploration of the wine-growing area, the methods and procedures of GIS were used in accordance with the principles for the use of GIS established in the OIV Resolution OIV-VITI 423-2012. For determination of spatial data, that is, parcels on which vineyards used to be for the purpose of modelling of potential historic viticultural parcels, archival maps from the Product Specification for the Protection of the Geographical Indication of the wine "Venčački Rizling" (Product specification No. 320-202/82-04 from 8. June 1982), and the documentation on vineyards from the Foundation of King Peter I Karadjordjevic in Oplenac were used. Spatial analysis was carried out for 667 micro-areas in which vineyards used to be in the territory of the current Oplenac wine-growing district.

Research of climate factors was carried out for the period 1988-2017, and the data from synoptic and climatological stations were interpolated by applying the successive correlation method (Cressman, 1959) on a regular grid with a horizontal resolution of 1 km, with the aim of spatial representation of climate characteristics. The modelled and spatially represented climate data were researched in accordance with the principles of OIV zonal resolution: Resol. OIV-VITI 423-2012. For modelling soil *terroir* factors, we processed soil data from previous research conducted by the Institute of Soil Science in Belgrade (Mrvić *et al.*, 2013) and obtained an estimate of soil loss (t ha^{-1} god^{-1}) by applying the USLE model (Wischmeier and Smith, 1978). To verify and confirm the data on modelled basic soil *terroir* factors from previous studies, we conducted additional soil analyses at four representative localities.

Laboratory analysis of wine quality and typicity parameters from representative vintages was conducted for wine made from *Chardonnay* and *Cabernet Sauvignon* grapes. A total of 25 quality and typicity parameters were analyzed using the WineScanTM Flex interferometer (merging of multiple light sources), which is based on Fourier transform infrared spectroscopy/infrared spectroscopy with Fourier transform (FTIR) technology (OIV/OENO 390/2010).

The application of the AHP and MGD methods, as well as the GIS technology, were used for the research, modelling and classification of the abiotic *terroir* factors, which in turn were used for the determination and classification of locations and micro-areas in relation to the favorability of the abiotic *terroir* factors.

The TOPSIS method and GIS technology were used to research, modelling, and ranking of anthropogenic *terroir* factors, which were used to determine and classify the vineyards in terms of the favorability of the anthropogenic *terroir* factors.

Further use of the GIS technology was aimed at modelling and determining small appellations, in particular: potential absolute, elite, and historic vineyard parcels, as well as potential organic wine-growing locations and micro-areas in the I (first) and II (second, more rigorous) levels of modelling.

The predictions on the quality and typicality of the wine, i.e. confirmation of data on the most important selected *terroir* factors and their impact on the quality and typicality of the wine, especially the wine with geographical indications, were made by applying the RFC method.

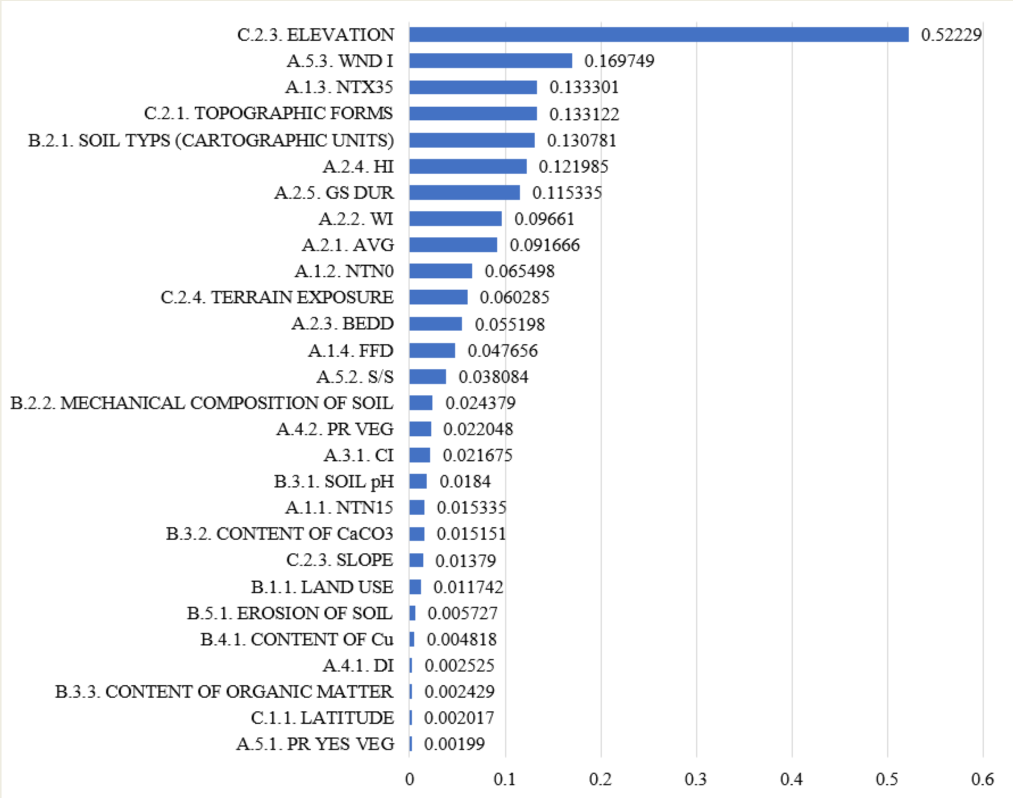
After the completion of the research and modelling results, and after the model validation, we developed an innovative scheme for a comprehensive characterization and classification of vineyards.

Results and Discussion

Modelling abiotic *terroir* factors

Modelling was performed by integrated application of the AHP method and GIS technology with three hierarchical criteria (general climate, soil and topographic *terroir* factors), 12 sub-criteria (*terroir* sub-factors) and 28 alternatives (basic *terroir* elements). At the first level of the hierarchy, the topographic *terroir* factor was selected as the most important criterion (general *terroir* factor). The next most important criteria, i.e., general *terroir* factors, were the soil and climate *terroir* factors. The consistency ratio determined in each case (CR) showed that there was a high consistency in the evaluation by the decision makers, and all importance ratings and rankings were confirmed as appropriate and valid.

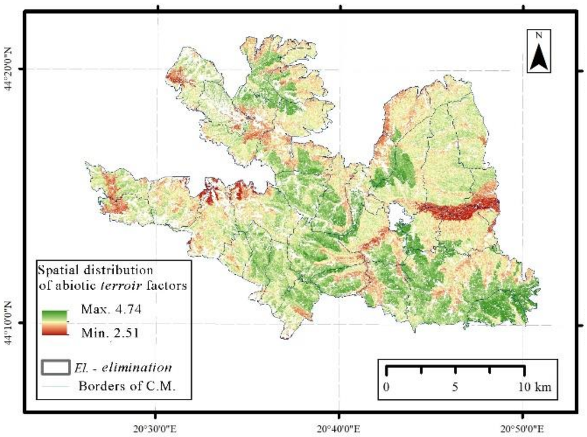
By applying the MGD method and determining the *q* value, the resulting factor detector showed that the highest *q* value is with the basic *terroir* element of altitude, and it can be concluded that this basic element has the highest importance among the studied abiotic *terroir* factors in terms of modelling the abiotic *terroir* factors (Graph 1).



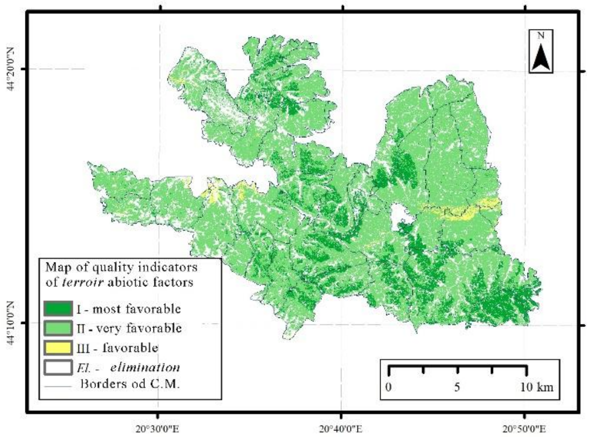
Graph 1 - Results of the factor detector for abiotic *terroir* basic elements

The risk detector of the MGD method was used to determine the average frequency of the basic *terroir* elements. The results of the risk detector show that in class I (most favourable), elevation as a basic *terroir* element has by far the highest frequency. On the positive side, of the 25 basic abiotic *terroir* elements, 15 elements in class V (least favourable) of the abiotic *terroir* factor quality indicators have no frequency at all (Table 1 in the Appendix).

By summing the reclassified favorability maps with the GIS technology, i.e. by summing the grid layers for all modelled basic *terroir* elements and for all modelled *terroir* subfactors and finally for the general *terroir* factors into a final sum map and by classifying them into equidistant classes of I (most favourable area), II, III, IV and V (unfavorable area) we obtained the final map of quality indicators for modelled abiotic *terroir* factors. The Oplenac wine-growing district has favourable abiotic *terroir* factors, and the obtained values of quality indicators for abiotic *terroir* factors ranged from minimum 2.51 (favourable class) to maximum 4.73 (most favourable class) (Map 1). The most favourable class (Class I) accounts for 14.16% of the area of the Oplenac wine-growing distict (Map 2).



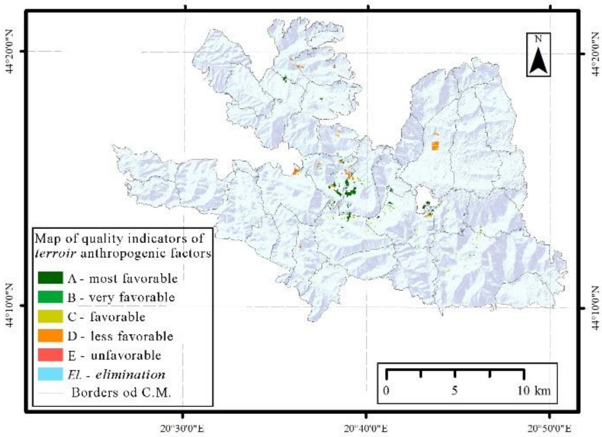
Map 1 - Spatial distribution of abiotic *terroir* factors



Map 2 - Map of quality indicators of abiotic *terroir* factors

Modelling of anthropogenic *terroir* factors

Based on research and modelling of anthropogenic *terroir* factors through application of the TOPSIS method, it was determined that the most significant anthropogenic factor is the alternative (anthropogenic *terroir* factor) vine variety. By connecting vectoral data from vineyards with class ranges of quality indicators of anthropogenic factors, we drafted the final map of anthropogenic *terroir* factors quality indicators, with favorability classes A (most favorable), B, C, D, and E (unfavorable). It was determined that, on the level of the entire wine-growing area, the share of the most favorable A class is 29.43% of the entire surface (111.20 ha) (Map 3).



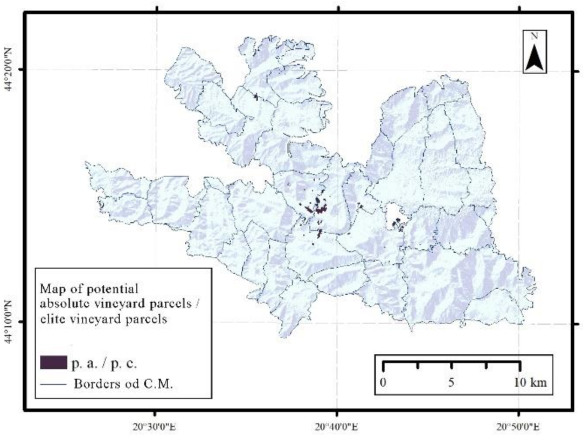
Map 3 – Map of anthropogenic *terroir* factors quality indicators

Modelling of small appellations

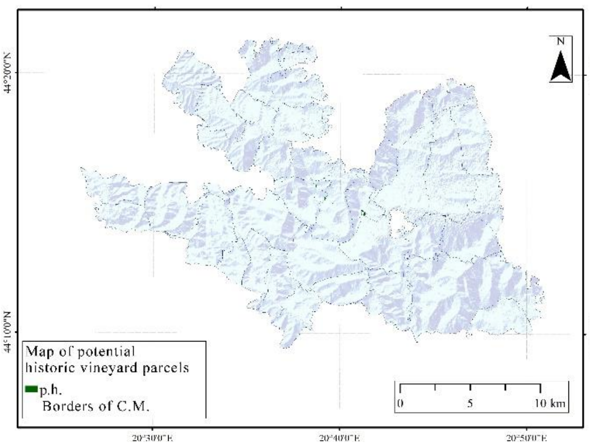
Modelling of absolute and elite vineyard parcels

By applying the GIS technology and selecting vineyards classified in class I (most favourable) of the modelled abiotic *terroir* factors and in class A (most favourable) of the modelled anthropogenic *terroir* factors, we created a map of potentially absolute vineyard parcels (p. a.), i.e., potentially elite vineyard parcels (p. e.). Of the total vineyards area of 282.03 ha, 91.18 ha are classified as potentially absolute (p. a.) and potentially elite (p. e.) vineyard parcels (Map 4).

By applying the GIS technology, i.e., by overlaying selected areas representing vineyards that meet the criteria for classification as p. a. or p. e., i.e., vineyards that meet the criteria for the traditional term for elite vineyard parcels and other legal conditions, and areas representing parcels that demonstrably had vineyards dedicated to the production of high quality grapes and wines, we created a map of potential historic vineyard parcels (p. i.). Of the total area of vineyards surveyed, 282.03 ha, 7.77 ha met the conditions for classification as p. i. (Map 5).



Map 4 - Map of potential absolute vineyard parcels / potential elite vineyard parcels



Map 5 - Map of potential historic vineyard parcels

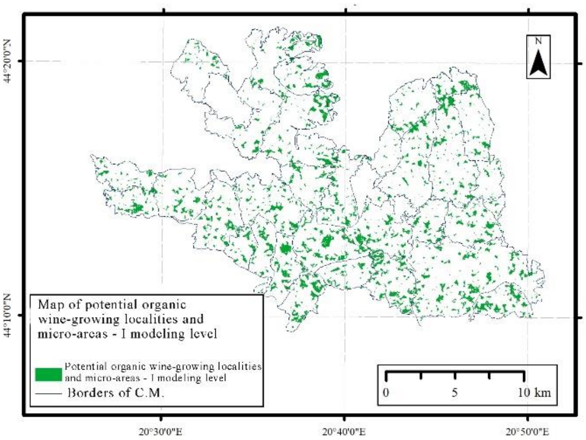
Modelling of potential organic wine-growing locations and micro-areas

I Modelling level

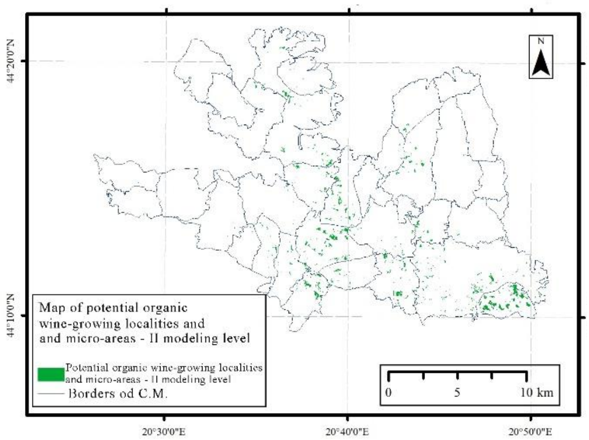
By creating buffer zones (using GIS technology) around the units at risk for organic cultivation, at a depth of 100 m around the potential pollutants, and by eliminating unfavourable soils that cannot be used for viticulture at the level of abiotic *terroir* factors, quality indicators I (most favourable) and II (very favourable), we selected potential organic wine-growing localities and micro-areas of the I modelling level (p. o. I) which totaled 3,658.16 ha (Map 6).

II Modelling level

At the II modelling level for determining the organic wine-growing locations and micro-areas, the same GIS operations were performed as at the previous modelling level, but in this case only operations in class I (most favourable) of the quality indicators for abiotic *terroir* factors were performed. From the total area of all vineyards, potential organic vineyard locations and micro-areas according to the II modelling level (p. o. II), 481.98 ha were selected (Map 7).



Map 6 - Map of potential organic wine-growing locations and micro-areas according to the I modelling level



Map 7 - Map of potential organic wine-growing locations and micro-areas according to the II modelling level

Model for prediction of quality and typicality of wine

By applying the RFC method, we selected two clusters representing common characteristics of the analysed quality and typicality parameters of wine of grapes of *Chardonnay* and *Cabernet Sauvignon* vine varieties. The obtained data, i.e. AIC, BIC and *silhouette* values, confirm the reliability of the model. The clustering of quality and typicity wine parameters confirmed the assumption that the studied quality and typicity wine parameters are grouped with respect to the predominant association of abiotic (cluster 1) versus anthropogenic *terroir* factors (cluster 2). In addition, application of the RFC method was used to determine the importance and ranking of examined quality and typicality wine parameters through use of the *Gini* index, wherein the dominant parameters that characterize the quality and typicality of wine were selected and correlated with the most important *terroir* factors determined through modelling of abiotic and anthropogenic *terroir* factors.

System (model) for comprehensive characterization and classification of vineyards

Based on the above modelling, a system (model) for comprehensive characterization and classification of vineyards was developed, in which vineyards are characterised based on two class groups for two basic models (modelling abiotic and anthropogenic *terroir* factors) and based on six class groups and marks within five thematic models (modelling small appellations and geographical indications for wine).

Conclusion

In order to evaluate the *terroir* factors with using the advanced technology, innovative multifactorial spatial models have been developed based on scientific expert research that allow to determine the contribution of the studied *terroir* factors to the successful cultivation of the vine and to the production of high quality wines typical of the studied wine-growing area, especially wines with geographical indications. Since the models developed are comprehensive and involve a multidisciplinary approach, we have developed two basic (for modelling and classifying abiotic and anthropogenic *terroir* factors) and three thematic models (for modelling small appellations), as well as a model for predicting the quality and typicality of wines that validates previous models and results obtained. The paper presents the spatial data of the final maps using the example of the Oplenac wine-growing district for the developed models supported by spatial research, where the localities, micro-areas and vineyards are presented in relevant favorability classes, i.e. in relation to relevant marks. Finally, we developed an innovative system for comprehensive characterization and classification of vineyards, based on classification of vineyards and their designation per eight different classifications for each of the developed viticultural-oenological models.

The obtained modelling data showed that there is high consistency with valuation by decision-makers, thus, all valuations of significance, i.e., rankings in models were confirmed as appropriate and valid. Therefore, the developed modelling ensures multifactorial and spatial procedures and methodologies for proving the connection between examined *terroir* factors and the quality and typicality of wines in accordance with the EU PDO/PGI system, and for potential protection of small appellations.

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Other sources

http://www.geodetector.cn/ - Geodetector

Appendix

Table 1 – Results of risk detector for abiotic basic *terroir* elements

Basic *terroir* elements	Favorability class				
	V	IV	III	II	I
A. 1. 1. NTN15			3.716552	3.65564	3.735621
A. 1. 2. NTN0	3.234000	3.652477	3.700285	3.793158	3.649085
A. 1. 3. NTX35	3.539651	3.766637	3.807786	3.607576	3.010778
A. 1. 4. FFD	3.483707	3.666108	3.697433	3.786948	3.717534
A. 2. 1. AVG		3.461709	3.750025	3.742464	3.47903
A. 2. 2. WI	3.051559	3.52062	3.770632	3.73291	3.484431
A. 2. 3. BEDD		3.454441	3.767311	3.687429	
A. 2. 4. HI	3.435747	3.652964	3.79528	3.759373	3.53842
A. 2. 5. GS DUR	3.459234	3.693722	3.773277	3.764157	3.483371
A. 3. 1. CI	3.225870	3.600385	3.731481	3.698123	3.69563
A. 4. 1. DI			3.719066	3.68573	
A. 4. 2. PR VEG		3.596517	3.590888	3.746721	3.719862
A. 5. 1. PR YES VEG			3.684472	3.715903	3.717127
A. 5. 2. O/Z		3.611316	3.577685	3.671491	3.78694
A. 5. 3. WND I	3.424475	3.633209	3.79132	3.833117	3.586522
B. 1. 1. Manner of soil use		3.685494	3.70121	3.790294	3.806086
B. 2. 1. Types of soil – cartographic units	3.489231	3.585675	3.737055	3.75909	3.810533
B. 2. 2. Mechanical soil composition		3.816748	3.709151	3.759384	3.626496
B. 3. 1. pH soil reaction			3.573458	3.677953	3.745755
B. 3. 2. $CaCO_3$ content		4.003857	3.700026	3.872904	3.778557
B. 3. 3. Organic matter content		3.70498	3.783877	3.689507	3.71927
B. 4. 1. Total copper content (Cu_T)			3.836583	3.741721	3.703161
B. 5. 1. Soil loss through water erosion		3.215821	3.480654	3.701701	3.712547
C. 1. 1. Geographic latitude				3.419524	3.709702
C. 2. 1. Topographic forms	3.585836	3.547378	3.679662	3.891818	3.807718
C. 2. 2. Elevation	3.056989	2.840018	3.554886	3.592399	3.936552
C. 2. 3. Terrain slope	3.700496	3.758795	3.719083	3.674789	3.639924
C. 2. 4. Terrain exposure	3.555627	3.668595	3.718752	3.751237	3.832794

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Examination of the influence of soil type on the yield and morphological parameters of *Mellisa officinalis*

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Abstract

Mellisa officinalis is a medicinal and spice plant known for centuries and is used in cooking and pharmacy. Although *M. officinalis* is easily adaptable to different habitat conditions, finding the most adequate agroecological conditions, primarily soil conditions, where this plant species can show the best productivity is important. In this regard, an experimental, potted production of *M. officinalis* was carried out in four different types of soil (arenosol, fluvisol, cambisol, chernozem) to examine which of the types best favors the yield of this plant species. A significant difference in the leaf yield of *M. officinalis* grown in different soil types was observed ($P<0.05$). The best leaf yield per plant was recorded in heavier soils (cambisol and chernozem) (1.56; 1.57 g), while in lighter soils (arenosol and fluvisol), the yield was significantly lower (0.92; 0.98 g). The research showed that of the tested soils, heavier soils (cambisol and chernozem), due to their favorable physical and chemical properties, have the most favorable effect on the growth and yield of *M. officinalis.*

Key words: arenosol, fluvisol, cambisol, chernozem, lemon balm

Introduction

Mellisa officinalis is a perennial herb that belongs to the Lamiaceae family. It is popularly known as motherwort, lemon balm, bee grass, etc., and has been used in culinary and traditional medicine for over 2000 years. Mainly, it is used for the treatment of the central nervous system and mental diseases, respiratory, cancer, and cardiovascular diseases. It is also used as a heart tonic, sleeping aid, memory enhancer and antidepressant (Ghazizadeh, 2021). Recent research indicates that *M. officinalis* exhibits numerous biological activities such as antimicrobial, hypoglycemic, hypolipidemic, anticancer, antidepressant, anxiolytic, antinociceptive, anti-inflammatory, antioxidant and spasmolytic properties (Miraj et al., 2016). Other phytochemical research confirmed the presence of volatile compounds of flavonoids, triterpenes, phenolic and dihydroxycinnamic acids (Mencherini, 2007).

M. officinalis has a highly branched root system with many lateral vascular roots that have great suction power. The tree is herbaceous, upright, 30 to 125 cm tall, semi-shrubby, and covered with small fine hairs. The leaves are egg-shaped to rhomboid, scaly, toothed around the edge, different lengths (2-8 cm), and widths (1.5-3.5 cm), which are attached to the stem by long petioles (1.5-3.5 cm). 6-10 compound flowers appear in the axils of the leaves in the form of shortened dichasial. The flowers are white, and pale pink and form from 1 to 4 nuts, and after flowering, an elongated egg-shaped, shiny, dark-brown grain of 1.5-2 mm in length is formed (Stepanović, 2011).

M. officinalis grows in the temperature range of 15 to 35 º and has high water requirements (500-600 mm) during the growing season (Saeb, 2012). The very physiology of the plant, primarily, a well-developed root system, makes this plant much more resistant to adverse environmental conditions (Turhan, 2006). However, research by Orcutt et al. (2000), indicate that biotic and abiotic factors can exert different influence on plant production. Soil type is an important abiotic factor that can exert a different influence on the growth and development of plants through the supply of water and necessary nutrients (Garg et al., 2012). Similarly, this is confirmed by the research of Radanović et al. (2006), who state that soil types and textural classes are closely related to the growth and development of medicinal plants. Similar research by Sari et al. (2002), on the mother plant, states that environmental factors, primarily soil factors, can have different effects on its productivity. However, since no detailed research was conducted to assess the effects of different types of soil on the productive properties of *M. officinalis*, the aim of this paper was to show the influence of four types of soil on the basic yield parameters and morphological characteristics of *M. officinalis*.

Material and Methods

Production of seedlings

Seedling production began at the end of January 2022 in the laboratory of the Department of Research and Development in Agriculture, in Belgrade, Serbia (44°49′ N, 20°28′ E). The motherwort (*M. officinalis*) seeds used in this experiment originated from the collection of the

Institute for the Study of Medicinal Plants "Dr. Josif Pančić", in Pančevo, Serbia (44° 52'20.0" N, 20°42'04.7" E). The sowing procedure itself was carried out according to the procedure described in the research by Mrđan et al., (2022). More precisely, the seeds were sown in styrofoam containers with 160 openings, previously filled with the substrate "Cultivo I SF" (Gramoflor, Romania) with the following performances declared by the manufacturer: granulation: 0 - 5 mm; nutrient content: NPK 18:10:20+Mg+me in the amount of 1 kg/m3, slow-acting fertilizer: RADIGEN®- Jost GmbH in the amount of 50 g/m3; hydrogel in the amount of 1 kg/m3.

After sowing, the sown pots are placed in a polyethylene tent (picture 1), (Grow Box), at air humidity of 40 to 60%; air temperature from 20°C to 25°C. Lighting was regulated using fluorescent tubes with a photoperiod of 12 h, while the substrate was maintained at a moderate humidity and temperature of 21±2 °C.

Air temperature and relative humidity in the Grow Box were monitored using a data logger (HAXO-8) and substrate temperatures using a thermometer (Testo 110).

Tempering process

With the appearance of the first true leaves at the end of February 2022, the containers were taken out of the polyethylene tent and were occasionally taken outside in order to adapt the seedlings to lower air temperatures, reduced relative humidity, and natural light radiation for a period of 3 weeks as recommended by Mrđan et al., (2022). After that, in mid-March, the plants were transplanted into styrofoam containers with 40 cells filled with the same substrate (Cultivo I SF) and then transferred to an unheated greenhouse, in 30% shade with an average daily T of 24±2 º. Seedlings were watered as needed. At the beginning of April, the containers were taken out of the greenhouse for adaptation for 3 weeks, and then the plants were transplanted into plastic pots (ø 13 cm).

Experimental design

The experiment was set up at the end of April according to the split-plot system with four replications, where soil type (F1 = 4 soil types) was the main factor. In general, 160 uniform seedlings were selected and transplanted into 160 pots filled with four different soil types (Table 1), of which each soil type had 40 pots.

Table 1. Physical and chemical characteristics of soil

Soil type	**pH**		**CaCO$_3$**	**Humus**	**N**	**P$_2$O$_2$**	**K$_2$O**	**Fine sand [%]**	**Coarse sand [%]**	**Powder [%]**	**Clay [%]**
	in KCl	in H$_2$O	**[%]**	**[%]**	**[%]**	**[mg/100 g]**	**[mg/100 g]**	0.02-0.2 [mm]	0.2-2 [mm]	0.02-0.002 [mm]	<0.002 [mm]
Arenosol (1)	7.99	8.55	19.08	0.40	0.044	1.39	2.00	67.79	28.96	1.1	2.31
Fluvisol (2)	7.49	8.08	14.10	1.28	0.110	3.99	7.29	66.07	27.91	5.7	1.89
Cambisol (3)	3.62	4.74	0.1	1.90	0.120	2.79	13.38	29.99	1.93	80.01	17.98
Chernozem (4)	**6.70**	**7.50**	**0.99**	**2.89**	**0.220**	**4.95**	**33.58**	27.62	0.31	31.98	39.02

The planted pots were placed on the experimental field of the Institute for the Study of Medicinal Plants "Dr. J. Pančić" in Pančevo, South Banat, Serbia (77 m above sea level; 44°52'18.9” N 20°42'09.0” E). The placed pots were in semi-shade, on a black "agro textile" foil that covers the soil in natural conditions. At the same time, the pots were irrigated using drippers with a flow rate of 1 liter per hour for each pot. Irrigation was done every two days for 20 minutes (Figure 2). Meteorological data during the experiment were collected from an automatic meteorological station, not far from the experimental field (Table 2).

Figures 1. and 2. *M. officinalis* in a grow box and on agro textile

Table 2. Meteorological parameters during the experiment (April-Jul 2022)

Climatic parameters	**April**	**Maj**	**Jun**	**Jul**
Temperature (°C)	17.9	27.0	31.3	32.8
Precipitation (mm)	57.2	34.9	23.5	72.0

Harvest, morphological and statistical analysis

After 180 days, in mid-July, with the appearance of the first flowers according to Singh et al. (2014), the plants were harvested and then their height (cm) was measured. After that, fresh and after drying at 105 °C to constant mass and absolute dry above-ground biomass (g/plant) were measured. In order to calculate the Radman yield, after drying, the leaves were separated from the stem (splitting) on a metal sieve with a 10 mm opening. The obtained results were statistically analyzed using SPSS software, where the obtained mean values were compared using Duncan's test ($p<0.05$).

Results and Discussion

The tested types of soil had different effects on all measured parameters (Table 3). This is explained by the research of Hassink (1996), in which it is stated that the texture of the soil exerts a great influence on the accumulation of organic matter, the water balance of Hook et al. (2000), and then on the availability of nutrients to plants (Brady, N. C. 1990). The types of soil in our research significantly influenced the height of *M. officinalis*. The highest average height was achieved by growing in soils with a heavier mechanical composition: chernozem and cambisol, while it was lower in soils with a lighter mechanical composition: arenosol and fluvisol (Table 3).

The average weight of fresh and dry above-ground biomass varied significantly due to differences in soil types, which at the same time manifested different influences on the average weight of leaves and branches in cultivated plants (table 3). The highest average weight of fresh and dry plants of *M. officinalis* was achieved by growing in heavier soils, cambisol and then in chernozem. More precisely, the average weight of fresh and dry plants was from 59.1% to 67.6% higher than in soils with a lighter mechanical composition (arenosol and fluvisol) (Table 3). Similar effects were achieved by the research of Moqbeli et al. (2011), when growing *M. officinalis* in different soil types.

More precisely, a significantly higher height, fresh and dry weight of the plant was obtained by cultivation in soils with a heavier mechanical composition, while the morphological parameters in the research of Gordanić et al. (2021), were significantly better, which is explained by its production in a commercial substrate. In the research of Kahkashan et al. (2016), by growing *Mentha arvensis* in different types of soils, the maximum yield was the same as in ours, in soils with a heavier mechanical composition.

Table 3. Average values of plant height and yield of *M. officinalis* grown in pots for 160 days

Treatment	Plant height (cm)	weight (FW) (g/plant)	weight (DW) (g/plant)	Leaf weight (g/plant)	Weight branches (g/plant)	Ratio branches:leaf
Arenosol (1)	38.8±2.02d	5.2±0.42d	1.39±0.41d	0.92±0.13c	0.46±0.25	2:1
Fluvisol (2)	39.5±1.94c	5.5±0.31c	1.42±0.53c	0.98±0.25b	0.42±0.34	2.1:0.9
Cambisol (3)	40.2±3.03b	6.7±0.36a	2.35±0.22b	1.56±0.33a	0.78±0.42	2:1
Chernozem (4)	43.4±2.75a	6.25±0.28b	2.05±0.14a	1.57±0.24a	0.47±0.34	2.3:0.7

*Mean values marked with the same lowercase letters within the same column do not differ significantly ($p < 0.05$).

The highest average weight of leaves and branches was in chernozem, while it was slightly lower in cambisol, with a significantly lower weight in arenosol and fluvisol (Table 3). Taking into account that the greatest use value of *M. officinalis* is the leaf, it is important to emphasize that the proportion of the leaf in relation to the stem differed considerably. The most favorable ratio (Table 3) was achieved by growing *M. officinalis* in chernozem, which, unlike others, can be attributed to more favorable physical and chemical characteristics of the soil (Table 1). Namely, favorable pH, high content of humus, and then optimal content of N, P, K in chernozem (table 1), played a major role in the dominant share of leaves of *M. officinalis* in contrast to the other three soils. Research by Hokkanen (2006) states that the physical and chemical properties of the soil play an important role in the water capacity of the soil. Similar research by Siemens et al. (2002), shows how soil resources (water, air, nutrients) can directly influence growth rate inhibition.

Accordingly, the textural properties of our soils were quite different: arenosol and fluvisol mostly contained fine sand (67.79 - 66.07%), cambisol mostly contained dust (50.01%), while chernozem had a similar proportion of dust (33.05%). and clay fractions (39.02%), which caused different moisture retention.

According to McCauley et al. (2005), these differences are very significant because soils with a more dominant share of clay materials (heavier soils) have better water capacity, which may be the main reason for the manifestation of positive characteristics of chernozem in this research. The dominant effect of chernozem was confirmed in similar research by Gordanić et al. (2022), who tested growing *A. ursinum* in different soil types.

Conclusion

The results of the study show that soil types have a great influence on the morphological parameters and yield of *M. officinalis*. This study shows that *M. officinalis* can be grown successfully as a potted crop using all four soil types tested. By growing *M. officinalis* in soils with a heavier mechanical composition (chernozem), the best morphological characteristics as well as a high-quality yield are achieved.

Acknowledgments

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Dietary risk assessment of diamide insecticides in peach fruits

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Abstract

In this study long-term and short-term dietary risk assessment of peach fruits treated with diamide insecticides, chlorantraniliprole and cyantraniliprole, was conducted. The evaluation was done by calculating the dietary risk quotient (HQ) and International Estimated Short-Term Intake (IESTI). In order to obtain estimated daily intake (EDI), residue data in treated fruits were generated from dissipation experiments. Plant protection products based on these two insecticides were applied at the rate recommended for the control of *Grapholita molesta*, at two localities in the Republic of Serbia, in peach orchards with the Royal gem cultivar. Fruit sampling was performed immediately after application till the end of the pre-harvest interval (PHI), and for the residue analysis, the QuEChERS-based method followed by HPLC was used. The chlorantraniliprole and cyantraniliprole residues in peach fruits dissipated with a half-life of 2.50 and 3.15 days. The amount of chlorantraniliprole in the peach fruits at the EU maximum residue level (1 mg/kg) was achieved seven days after the application (0.95 mg/kg), while cyantraniliprole was at the MRL (1.5 mg/kg), immediately after the drying of the deposit. For the EDI value of chlorantraniliprole and cyantraniliprole, results obtained from the field experiments were multiplied by the average consumption rate (16.7 g/day) and divided by the mean weight of an adult (70.8 kg). Finally, obtained results indicate low risk from chlorantraniliprole and cyantraniliprole residues in the peach, thus fruits can be safely consumed.

Key words: Risk assessment, Chlorantraniliprole, Cyantraniliprole, Peach fruits

Introduction

As a source of minerals, vitamins, and other nutrients, fruit represents an essential part of a healthy diet. However, due to the presence of a wide range of pests, fruit production requires chemical protection. Intensive and/or inadequate use of pesticides leads to the presence of pesticides in fruits, causing side effects on human health. To overcome these problems, nowadays, good agricultural production implies the use of pesticides with more convenient ecotoxicological properties (Lazić et al., 2012) and a shorter pre-harvest interval (PHI).

When it comes to peach production, one of the most significant and destructive pests is Grapholita molesta (Busck, 1916) and it is regularly found in peach and quince orchards (Stamenković 2005). Damages caused by *G. molesta* have an impact on overall plant conditions, reduce fruit quality and create economic losses. Recently, for the control of this pest, diamides insecticides, chlorantraniliprole and cyantraniliprole showed to be effective substances. However, only a few studies have reported the detection and dissipation of these insecticides in peaches (Žunić et al., 2020).

Despite the necessity for pesticide use, the risks related to their residues in the human body and the environment are being established. Even though very strict regulation processes during pesticide registration, with minimal impact on human health and the environment, are developed, serious concern have been raised about health risks resulting from occupational exposure to pesticides and from residues in food and drinking water (Damalas and Eleftherohorinos, 2011).

Thus, it is still required to evaluate the adverse health effects of pesticides through risk assessment. This is particularly important for food mainly consumed fresh, such as fruit.

Pesticide residue intake through the diet can be predicted with different degrees of accuracy, from "Crude estimation" with theoretical maximum daily intake (TMDI), to "Best estimation" using estimated daily intake (EDI) (UNEP/FAO/WHO, 1989). Risk assessment is based on a comparison of potential long- and short-term dietary intake of pesticides with two established parameters of hazard characterization, Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD). Long-term hazard implies a comparison of the Theoretical Maximum Daily Intake (TMDI) to the ADI of the pesticide, where the TMDI is based on assumption that food products consumed over the lifetime contain residues at the MRL level. It is calculated using the MRL and the average daily consumption of food for which an MRL has been established (Osman, 2011). For the calculation of hazard risk (HR), EDI values take into account residues in food at the level of median residue values from trials, thus including more factors and providing better estimation.

Short-term dietary exposure to pesticides is based on the comparison of International Estimated Short-Term Intake (IESTI) to ARfD. The calculation of IESTI requires data from field trials (Struciński et al., 2006).

In this study, the supervised field experiments were carried out to study the persistence, dissipation and risk of the consumption of peaches treated with chlorantraniliprole and cyantraniliprole was assessed.

Materials and Methods

Dissipation studies

For the dissipation studies field trials were conducted at two localities in the Republic of Serbia, according to EPPO methods (Žunić et al., 2020), in peach orchards under the Royal gem cultivar. Briefly, plant protection products based on cyantraniliprole (100 g a. i./l, SE) and chlorantraniliprole (200 g a. i./l, SC) were foliar applied at the recommended rate (0.6 and 0.2 l/ha, respectively) for the control of *G. molesta,* when the plants were in the BBCH 74 phenophase. Fruit sampling was performed daily during the PHI, while for the residue analysis, the QuEChERS-based method followed by HPLC was used. Estimated PHI for chlorantraniliprole and cyantraniliprole in the Republic of Serbia is 14 and 7 days, respectively.

Dietary risk assessment

In this study, HQ is obtained by dividing EDI (mg/kg bw/day) by the relevant ADI (mg/kg bw/day) value (Łozowicka et al., 2013). For the estimated daily intake of chlorantraniliprole and cyantraniliprole, residues (mg/kg) obtained from the field experiments were multiplied by the average consumption rate (16.7 g/day) and divided by the mean weight of an adult (70.8 kg). HQ describes the risk characterization of a single chemical, while the hazard index (HI) is used for a mixture of chemicals (EPA, 1986), i.e. the sum of all component chemicals' HQs constitutes the hazard index (HI) for the whole pesticide mixture's effect.

The long-term risk assessment of intakes and HQ is calculated as followed (Ambrus et al., 2023):

$$EDI = \frac{concentration\ of\ pesticide\ from\ field\ trial\ \left(\frac{mg}{kg}\right) * food\ consumption\ (\frac{kg}{day})}{\text{body weight of an adult (kg)}}$$

$$HQ = \frac{\text{EDI}}{\text{ADI}} \qquad \text{HI} = \sum \text{HQ}$$

Short-term dietary exposure to insecticides is evaluated through International Estimated Short Term Intake (IESTI), and it is calculated as followed using single-day consumption data for the 97.5 of food intake and the highest residue (HR) of the edible part of the crop observed in supervised field trials (Struciński et al., 2006).

IESTI = HR × 97.5% food intake

Results and Discussion

A dietary risk assessment was conducted based on the results of the dissipation experiment (Žunić et al., 2020). The dissipation dynamic of chlorantraniliprole and cyantraniliprole dissipation in peach fruits followed first-order kinetics with a half-life of 2.50 and 3.15 days. The long-term risk was evaluated by calculating (HQ) using residue data obtained in treated fruits immediately after application till the end of PHI and ADI. ADI value of chlorantraniliprole is 1.58 mg/kg bw/day (EFSA, 2012), while the toxicological profile of cyantraniliprole was assessed in the framework of the EU pesticides peer review under Regulation (EC) No 1107/2009, and based on these data an acceptable daily intake (ADI) is 0.01 mg/kg body weight (bw) per day. Results are shown in Tables 1 and 2.

HQ values for chlorantraniliprole in peach fruits range between 0.0006 immediately after insecticide application, and 0.00003 at the end of PHI. A similar situation occurred in the experiment with cyantraniliprole, where HQ ranged from 0.00034 to 0.00003. Numerically, HQ of >1, indicates that could be an unacceptable risk from chlorantraniliprole and cyantraniliprole residues present in the peach fruits. If the HQ is <0.1 the risk is low, while the values between 0.1 and 1.0, indicate moderate risk (Sánchez-Bayo et al. 2002). The results obtained in this study indicate that peach fruit treated with chlorantraniliprole and cyantraniliprole are safe for consumption.

Table 1. Dietary risk assessment of chlorantraniliprole in peach fruits

average peach consumption per adults per day (kg/day)	average adults body weight	Days after treatment	Maximum residue (mg/kg)	EDI (mg/kg/bw/day)	ADI (mg/kg/bw/day)	Risk quotient (RQ)	ARfD (mg/kg/bw)
0.0167	70.8	0	2.32	0.00055	1.56	0.00035	n.n.
0.0167	70.8	1	2.04	0.00048	1.56	0.00031	n.n.
0.0167	70.8	3	1.73	0.00041	1.56	0.00026	n.n.
0.0167	70.8	5	1.32	0.00031	1.56	0.00020	n.n.
0.0167	70.8	7	0.95	0.00022	1.56	0.00014	n.n.
0.0167	70.8	9	0.51	0.00012	1.56	0.00008	n.n.
0.0167	70.8	11	0.23	0.00005	1.56	0.00003	n.n.
0.0167	70.8	13	0.10	0.00002	1.56	0.00002	n.n.
0.0167	70.8	15	0.12	0.00003	1.56	0.00002	n.n.

n.n. not necessary

Table 2. Dietary risk assessment of cyantraniliprole in peach fruits

average peach consumption per adults per day (kg/day)	average adults body weight	Days after treatment	Maximum residue (mg/kg)	EDI (mg/kg/bw/day)	ADI (mg/kg/bw/day)	Risk quotient (RQ)	ARfD (mg/kg/bw)
0.0167	70.8	0	1.46	0,00034	0.01	0.03444	Acute risk assessment not required as an ARfD is not necessary (EFSA, 2014).
0.0167	70.8	1	1,32	0,00031	0.01	0.03114	
0.0167	70.8	2	1.2	0,00028	0.01	0.02831	
0.0167	70.8	3	0.65	0,00015	0.01	0.01533	
0.0167	70.8	4	0.46	0,00011	0.01	0.01085	
0.0167	70.8	5	0.39	0,00009	0.01	0.00920	
0.0167	70.8	6	0.24	0,00006	0.01	0.00566	
0.0167	70.8	7	0.19	0,00004	0.01	0.00448	
0.0167	70.8	8	0.13	0,00003	0.01	0.00307	

An acute reference dose (ARfD) is the estimate of the amount of a substance in food or drinking water, expressed on a milligram per kilogram body weight basis. This quantity of a substance can be ingested over a short time, usually in one meal or during one day, without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation. ARfD was not established for chlorantraniliprole and cyantraniliprole, as they are of low acute toxicity, and did not demonstrate evidence of a genotoxic, neurotoxic, or reproductive/developmental toxicity potential after a single dose. Despite this, short-term dietary risk to diamide insecticides is expressed as IESTI, with obtained values of 0.038 for chlorantraniliprole and 0.024 for cyantraniliprole.

Studies of risk assessment of chlorantraniliprole and cyantraniliprole in other agricultural products show a hazard index of less than 1 (Paramasivam, 2021; Saraswati et al., 2022).

Conclusion

The pattern of chlorantraniliprole and cyantraniliprole dissipation in peach fruits was best fitted to first-order kinetics with a half-life of 2.50 and 3.15 days. Based on these data dietary risk of the applied insecticides was assessed. According to results obtained for long- and short-term dietary risk assessment, peach fruits can be safely consumed after insecticide application in the doses recommended for effective control of *Grapholita molesta*.

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Morphological and quality attributes of selected autochthonous apple genotypes from Serbia

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Abstract

Apple is one of the most grown fruits in the world. Traditional apple genotypes represent an important resource that ensures the sustainability of apple production and high diversity of available genotypes. The aim of this study was to analyze the fruit quality and sensory characteristics of 5 autochthonous apple genotypes from Serbia, in order to determine their potential on the market for fresh consumption and processing industry. In order to determine the fruit visual characteristics, morphological traits were studied (fruit ground and over color, percentage and pattern of over color, greasiness of the skin, fruit weight, length and height and fruit shape). In addition, length of the fruit stalk and fruit firmness were determined, as important characteristics for fruit storability. Among the biochemical parameters, total soluble solids and titratable acidity were determined. Sensory analysis was performed for the following traits: attractiveness, taste, aroma, juiciness and astringency. The highest potential for fresh consumption showed ‘Šećeruša’ and ‘Čegarača’ genotypes. Genotype ‘Đeregarka’ is more suitable for processing industry due to its high content of organic acid. ‘Crvena Debelokorka’ had low taste and aroma ratings (partially due to high astringency), but also the highest fruit firmness, which can result in good storability. The analyzed genotypes showed diverse quality traits and could find their place in the market and in the economy of the small farmers, but also in breeding programs focused on quality and diversity. They should be preserved as an important genetic resource for the sustainability of agriculture.

Key words: apple, traditional genotype, morphological traits, soluble solids, sensory analysis

Introduction

Apple (*Malus domestica* Borkh.) is one of the most cultivated species of fruit trees in the world. Apple production is increasing worldwide and total production averaged 88026492 tones (2017-2021). More than half of the world's apple is produced in Asia. The largest producer is China, which produces almost half of the apple fruit, followed by the USA and Turkey. A positive trend in apple production is also observed in Serbia and the five-year (2017-2021) average of apple production in Serbia -was 468258 tons (FAOSTAT, 2021).

Apple production is influenced by various abiotic and biotic factors. Apples are susceptible to a wide range of pathogens, such as apple scab, powdery mildew, tree root rot disease and fire blight. Among abiotic factors drought and frost stress, low spring temperatures and hailstorms are the most challenging. These factors affect both, fruit yield and fruit quality. Climate change also induces higher risk of biotic stress as unpredictable changes can affect disease and pest management (Moinina et al., 2019). Responses to these challenges depend heavily on traditional varieties, local populations and wild relatives of plant genetic resources for food and agriculture (Bramel and Volk, 2019). They can be important resources for resistance towards different abiotic and biotic stress factors (Balaž et al., 2017; Marić et al., 2016; Mihaljević et al., 2021). Traditional genotypes are often characterized by higher polyphenol content and antioxidant capacity compared to modern cultivars (Lončarić et al., 2021; Preti and Tarola, 2021), which are desirable components from the perspective of nutritional value and importance for human health, as well as beneficial against pathogen attack. The quality of the apple fruit presents complex interaction of morphological and biochemical parameters that are important for consumers. Morphological parameters such as color and fruit size are the first ones which consumers notice. Most consumers prefer red or green fruits (Benković-Lačić et al., 2022; Drkenda et al., 2021), but regarding the fruit size, opinions are different and range from preference for big apples (Drkenda et al., 2021), while another group of consumers prefers small or middle-sized fruits (Benković-Lačić et al., 2022). In addition, fruits should be without damage since the presence of changed color or texture does not look attractive to the consumers. Regarding fruit taste, sugars and organic acids are the most important compounds that determine the subjective perception of the fruit taste. Traditional apple genotypes have higher sugar and lower acid content in compare to modern apple genotypes, which makes them more attractive for both, fresh consumption and processing (Begić-Akagić et al., 2014). Aroma is also important quality attribute of the apple fruits that can affect the perception of the taste. It depends on the content of alcohol, esters, aldehydes, ketones and ethers (Espino-Díaz et al., 2016). The nutritional value of apple fruit also depends on the components such as vitamin C, carotenoids, flavonoids that are important for the antioxidative defense. Due to these phytonutrients, apples have antimicrobial, antiproliferative, anti-inflammatory, cardio-protective and other beneficial effects on human health (Seenivasan et al., 2022). The aim of this study was to analyze the fruit quality and sensory characteristics of 5 autochthonous apple genotypes, in order to determine their potential in the market for fresh consumption and the processing industry.

Material and Methods

The fruits of 5 autochthonous apple genotypes (‘Čegarača’, ‘Demirka’, ‘Đeregarka’, ‘Crvena Debelokorka’ and ‘Šećeruša’) were used for this study (Photo 1). The genotypes are part of the fruit collection of traditional apple genotypes of the Experimental Station »Radmilovac« of the Faculty of Agriculture, University of Belgrade. The apple trees are planted at 4 m ×1.5 m distance and grown in a slender spindle training system. Samples were collected from 3 trees per genotype and 10 fruits were collected from each tree. Fruits were in the stage of physiological maturity and stored for two months, without refrigerator in basement room, until analysis.

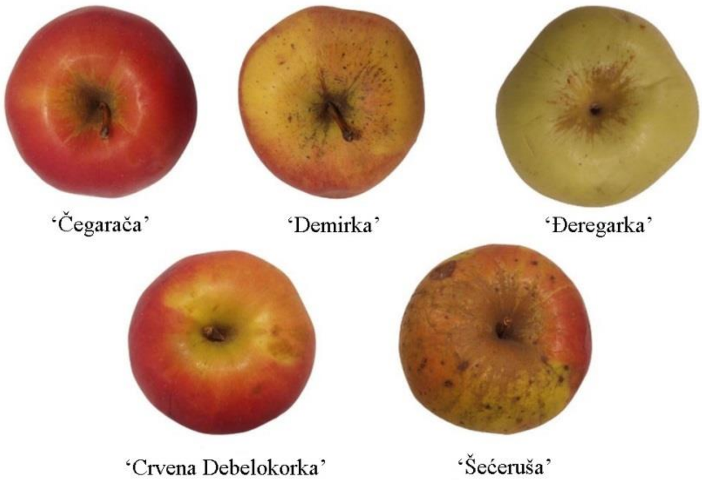


Photo 1. The fruits of the analyzed apple genotypes

The morphological characteristics of the fruits were studied: ground and over color of the fruit, distribution and percentage of the over color, greasiness of the skin, fruits shape, length of the fruit stalk, fruit weight (g), width and length (mm) and firmness of the fruit (kg/cm^2). In order to determine distribution of over color, greasiness of the skin, fruit shape and length of the fruit stalk, UPOV descriptor for apple was used (UPOV, 2005). Pattern of over color could be absent or present in different forms: solid flush, solid flush with weakly defined stripes, solid flush with strongly defined stripes, weakly defined flush with strongly defined stripes and flushed striped and mottled. Greasiness of the skin was rated on the following scale: absent or weak, moderate and strong. Fruit shape scale was used, with 7 different fruit shapes: conical, cylindrical, cylindrical waisted, ellipsoid, globose, obolid or ovoid. Regarding the length of the fruit stalk, cultivars are classified in 5 categories, based on the same descriptor: very short, short, medium, long and very long fruit stalk.

Fruit firmness was measured using a hand penetrometer FHT-1122 (Landek Instruments, China). Among the biochemical characteristics, the content of total soluble solids (°Brix) and the content of organic acids (%) were determined. Total soluble solids (TSS) were determined using HI96801 refractometer (Hanna Instruments, USA), while the total titratable acidity (TTA) was analyzed by the titration method (Tyl and Sadler, 2017). The sensory properties of the fruits were determined by scoring by 3 panelists, which evaluated the appearance, taste, aroma, juiciness and astringency of the fruit. Descriptive statistics (mean value ± standard error) were performed using the Sigma Plot program (version 11.0). Sensory analysis data and fruit firmness data were standardized (0-100%) and presented in the form of a radar plot.

Results and Discussion

The apples analyzed in this study had yellow, green or green-yellow ground color (Table 1). Red cover color was present in genotypes ‘Čegarača’, ‘Demirka’ and ‘Crvena Debelokorka’, while genotype ‘Šećeruša’ had pale red color. The cover color was dispersed in solid flush pattern. The fruits of ‘Čegarača’, ‘Demirka’ and ‘Crvena Debelokorka’ were characterized with high cover color (65-70%), while ‘Šećeruša’ had only 30% of over color. Only genotype ‘Đeregarka’ did not have any over color and was completely green. Greasiness of the skin was strong or absent in examined genotypes.

Table 1. Morphological parameters (ground color, over color, pattern and percentage of the over color and skin greasiness) of analyzed apple genotypes

Genotype	**Ground colour**	**Over colour**	**Pattern of over colour**	**Percentage of the over colour (%)**	**Greasiness of the skin**
‘Čegarača’	Green-yellow	Red	Solid flush	65	Absent
‘Demirka’	Yellow	Red	Solid flush	70	Absent
‘Đeregarka’	Green	Absent	Absent	0	Strong
‘Crvena Debelokorka’	Yellow	Red	Solid flush	70	Strong
‘Šećeruša’	Yellow	Pale red	Solid flush	30	Absent

The shape of the fruits was globose, conic or obloid, depending of the genotype (Table 2). The length of the fruit stalk was short (‘Đeregarka’, ‘Crvena Debelokorka’ and ‘Šećeruša’) or medium (‘Čegarača’ and ‘Demirka’). The fruits with the highest weight, height and width were genotypes ‘Šećeruša’ and ‘Demirka’, while the lightest fruits had ‘Crvena Debelokorka’. The lowest fruit height and width were present in the genotype ‘Đeregarka’.

Table 2. Morphological parameters (fruit shape, length of the fruit stalk, fruit weight, fruit height and width) of analyzed apple genotypes

Genotype	Fruit shape	Length of the fruit stalk	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)
‘Čegarača’	Globose-obloid	Medium	180.47 ± 7.31	63.4 ± 0.92	75.57 ± 1.19
‘Demirka’	Conic	Medium	210.50 ± 11.27	68.43 ± 0.78	78.87 ± 1.56
‘Đeregarka’	Obloid	Short	110.44 ± 23.97	54.23 ± 3.22	64.48 ± 4.22
‘Crvena Debelokorka’	Conic	Short	101.89 ± 3.48	57.93 ± 1.27	64.73 ± 0.56
‘Šećeruša’	Globose	Short	227.09 ± 19.66	67.62 ± 2.27	82.6 ± 2.90

Biochemical analysis related to total soluble solids showed the highest content in the ‘Crvena Debelokorka’ genotype, while ‘Đeregarka’ genotype had the lowest sugar and the highest acid content (Table 3). The lowest total titratable acidity was present in genotype ‘Čegarača’. The firmest fruits had the genotype ‘Crvena Debelokorka’, while the lowest fruit firmness was recorded in the genotypes ‘Demirka’ and ‘Šećeruša’.

Table 3. Biochemical parameters (total soluble solids and titratable acidity) and fruit firmness of analyzed apple genotypes

Genotype	Total soluble solids (°Brix)	Total titratable acidity (%)	Fruit firmness (kg/cm^2)
‘Čegarača’	14.90 ± 0.15	0.69 ±0.04	4.60 ± 0.25
‘Demirka’	16.00 ± 0.20	0.73 ±0.03	3.33 ± 0.33
‘Đeregarka’	14.80 ± 0.23	1.18 ±0.04	4.88 ± 0.75
‘Crvena Debelokorka	18.23 ±0.19	0.81 ±0.04	6.79 ± 0.55
‘Šećeruša’	16.67 ±0.47	0.80 ±0.05	3.41 ± 0.40

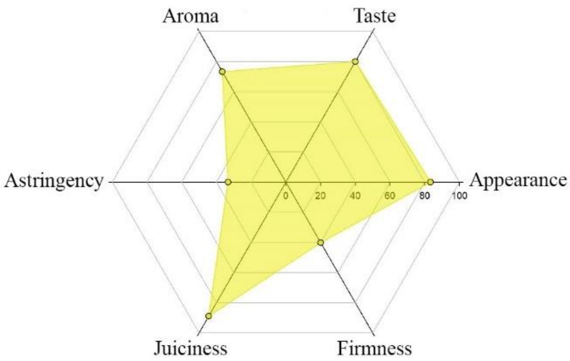
Sensory analysis showed that the best appearance had genotype ‘Đeregarka’ (Table 4), but also the lowest taste and aroma scores. A low taste and aroma rating were also present in genotype

'Crvena Debelokorka', which had the highest astringency. In contrast, the genotype 'Čegarača' had high taste and aroma rating, and the highest juiciness. The genotypes 'Demirka' and 'Šećeruša' also had good taste and aroma ratings, and satisfactory juiciness scores.

Table 4. Sensory analysis (appearance, taste, aroma, juiciness and astringency) of analyzed apple genotypes

Genotype	Appearance (1-10)	Taste (1-10)	Aroma (1-10)	Juiciness (1-3)	Astringency (1-3)
'Čegarača'	3.67 ± 0.33	8.33 ± 0.33	9.67 ± 0.33	2.5 ± 0.29	1.00 ± 0.00
'Demirka'	8.00 ± 0.58	7.33 ± 0.33	8.33± 0.33	2.00 ± 0.00	1.00 ± 0.00
'Đeregarka'	8.33 ± 0.33	2.33 ± 0.33	2.00 ± 0.00	2.33 ± 0.33	1.00 ±0.00
'Crvena Debelokorka'	5.33 ± 0.3	3.00 ± 0.58	2.67 ± 0.33	1.00 ± 0.00	2.33 ± 0.33
'Šećeruša'	8.00 ± 0.33	8.5 ± 1.00	7.5 ± 0.33	2.5 ± 0.33	1.00 ± 0.00

Based on these results, two of the most perspective genotypes 'Šećeruša' and 'Čegarača' (Figure 2) were presented by radar plots which highlighted their sensory attributes.



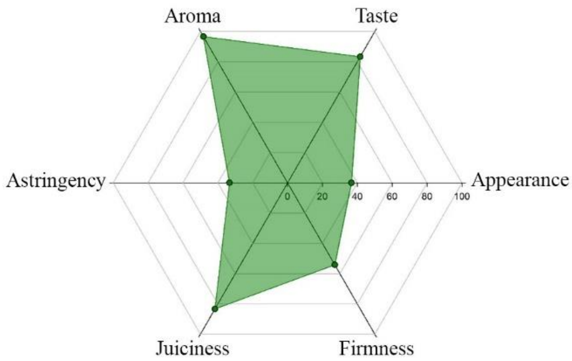


Figure 2. Radar plot of two selected genotypes - 'Šećeruša' (yellow) and 'Čegarača' (green)

Disccusion

Most of the analyzed apple genotypes had red over color, which is considered the most attractive (Dumanoglu et al., 2018) as consumers assume that red fruits are sweeter and have better overall quality (Musacchi and Serra, 2018). Therefore, breeders usually aim to produce apples with completely red or predominantly red fruits, with a high proportion of the over color. Fruit color is determined by environmental and genetic factors, and exhibits high heritability, resulting in genotypes with a higher degree of red color (Zheng et al., 2020). From this point of view, our genotypes 'Demirka' or 'Crvena Debelokorka' are promising and interesting for breeding programs. The genotype 'Šećeruša' had a pale red over color, which is becoming more popular worldwide, and even overcoming the popularity of the red over color. Due to the pale red blush, this genotype could be also interesting for future breeding programs (Musacchi and Serra, 2018). However, green-yellow is considered the most attractive ground color by most of the consumers (Dumanoglu et al., 2018). This ground color is present in the genotype ‘Čegarača’. Non-red genotypes (green and yellow color) should have other desirable properties in order to overcome the color unattractiveness and to be more appreciated by consumers (Normann et al., 2019). ‘Čegarača’ was also characterized by the most uneven fruits, which had variable fruit shape: globose, obloid and globose-obloid. On the contrary, genotype ‘Đeregarka’had a uniform fruit shape.

All studied genotypes belonged to the group of medium or small apple fruits, which is in the commercial production considered as a disadvantage. However, some research showed that consumers in this geographical area prefer middle-sized (Dumanoglu et al., 2018) or small fruits (Benković-Lačić et al., 2022), considering that those fruits are grown in non-intensive agricultural systems and healthier (Skreli and Imami, 2012). Length of the fruit stalk was medium in genotypes ‘Čegarača’ and ‘Demirka’, while the other genotypes had a short length of the stalk. Genotypes with longer fruit stalk are more desirable in commercial fruit orchards since these fruits are more difficult to detach from the tree and the harvest is much easier in compare to short stalk genotypes. Fruits with long stalk are usually collected with the stalk, while the short stalk is easy to be pulled out of the fruit during harvest, which affects the storability of the fruit (Radović, 2022). Salkić et al. (2017) reported similar results to the ones obtained in this study, where traditional genotypes with long stalk are rare, which presents their disadvantage regarding their use in production and storability.

Apple fruits differ in soluble solids content based on genotype, but also on environmental conditions. Soluble solids content is the mostly determined by sugar content, which affect the most fruit taste and nutritional value. The sweetness of the fruit is determined not just by the overall sugar content, but also the presence and the ratio of the individual sugars (glucose, fructose, sucrose and sorbitol). Our results showed that fruits of ‘Crvena Debelokorka’ had the highest value of total soluble solids and medium level of acids content. High soluble solids content can affect fruit taste since the most of the consumers prefer sour-sweet apples (Benković-Lačić et al., 2022). The optimal ratio is present in the fruit ‘Šećeruša’ since it had relatively high soluble solids and moderate acid content, so it is expected that fruits of ‘Šećeruša’ would have a good sensory property.

Genotype ‘Đeregarka’ had the undesirable TSS/TTA ratio, with high titratable acidity and low soluble sugars, which affects fruit taste. Still, high content of organic acids indicates the potential of this genotype for long storage, as well as in the processing industry since acid content is important for the quality of the products and their sanitary safety. Literature data show a correlation between fruit color and titratable acidity, since non-red genotypes have higher titratable acidity in comparison to red ones (Kumar et al., 2018), which is also the result of this study related to 'Đeregarka' genotype.

Fruit firmness was the highest in 'Crvena Debelokorka', and this trait is important for storability of the fruit, and in a close relation to the other fruit rheological properties. Low firmness of fruit during storage time is related to fruit transpiration and water loss, which lead to changes in fruit properties (Fathizadeh et al., 2021).

Appearance fruit score in sensory analysis showed that consumers from Serbia considered green genotype 'Đeregarka' (with no over color), as the most attractive. The lowest taste and aroma ratings were recorded in this genotype. Its fruits had high titratable acidity and low soluble solids content, which indicates that taste was perceived as sour and unpleasant. Still, juiciness of the fruits was rated as good, which in combination with high sourness and high acid content can determine this genotype potential for processing industry. 'Demirka' also was rated as genotype with attractive fruits because of its red color, but also the genotype 'Šećeruša', which confirms the conclusion that pale red fruits are becoming more popular among consumers (Musacchi and Serra, 2018). On the other side, the fruit quality of 'Šećeruša', characterized with high soluble solids and moderate acid content, was confirmed by sensory analysis.

On the contrary, genotypes 'Čegarača' and 'Crvena Debelokorka' had the least attractive fruits. The low attractiveness of the "Čegarača" fruit could be caused by the fact that fruits were visually unequal - fruit shape varied between globose, obloid and globose-obloid. Despite the intense red color and small sized fruits of 'Crvena Debelokorka', low attractiveness of fruits was related with fruit damage by apple scab (*Venturia inaequalis*), which affected consumers preference (Kelley et al., 2010). Genotypes sensitive to apple demand higher expenses of production and does not suit consumers' preference for less treated apples. 'Crvena Debelokorka' had high soluble solids in ratio to acids, which imply the taste is too sweet, without desirable sourness (Benković-Lačić et al., 2022). Also, aroma of fruits was low, which possibly affected the perception of taste since (Charles et al., 2019). Also, the astringency of fruits was high. Astringency is not just a problem for fresh fruit consumption, but also for the processing industry since it makes juices unpleasant and slightly bitter (Heinmaa et al., 2017). In combination with apple scab sensitivity, this genotype is not suitable for wider production.

Conlusions

Analyzed genotypes showed different morphological, biochemical and sensory attributes. Two genotypes have especially high potential as the genotypes for fresh consumption – 'Šećeruša' and 'Čegarača'. Regarding the fruit attractiveness, genotype 'Šećeruša' is the most interesting because of the new market demands toward pale red fruits. The fruits of genotype 'Đeregarka' are not suitable for fresh consumption, but could be interesting for the processing industry due to high organic acid content. This genotype has also very good potential for long term storability, which is an important quality trait. It is also important to note it was the most attractive genotype for consumers, although it is widespread opinion of preference for fruits with intense red color. It seems that consumers rated this genotype based on fruit size and irregular shape, as the proof of the fruit authenticity and traditional origin. Genotype 'Crvena Debelokorka' had high astringency and the lowest ratings regarding taste and aroma. Also, it is sensitive to apple scab, which makes it unsuitable for wider consumption. Still, it is important to notice that local communities maintained this genotype during the time, which implies it can show full potential in different agroecological conditions. All analyzed genotypes are saved in the fruit orchard collection at the Faculty of Agriculture, which presents an important step in the *ex situ* conservation of these genotypes. The management of these plant genetic resources should also include *in situ* conservation and return of these fruits on the markets.

Acknowledgements

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Influences of *Bacilus suptilis* and *Trichoderma harzianum* to productivity and fruits quality of strawberry cultivar 'Clery'

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Abstract

In the study were examined an influence of different microorganisms to improve yield and quality of fruits strawberry cultivar ‘Clery’. The microbiological preparations were applied by drip irrigation system in doses of 5 l/ha, two and four weeks after planting, and also, next spring four and two weeks from the expected beginning of blooming. The following properties of productivity were analyzed: the number of crowns per plant, diameter of crowns, the number of inflorescences per crowns, the number of fruits per inflorescence, the total number of fruits per plants and the yield per plants (total and separately per harvesting in kg). According UPOV Code for strawberry the following phenological properties were studied. Plants treated with *T. harzianum* had significantly higher and numbers of inflorescences per crowns. A percentage of harvested fruits in first harvest, in regard to total fruits per plants, were between 19.5% (control plants) to 25.3% (*B. suptilis*). In the second harvest, to all treatments were recorded an increased yield of harvested fruits compared to first harvest. A total yield of marketable fruits per plants ranged between 0.38 kg (control) to 0.56 kg (*T. harzianum*). The highest content of TSS had fruits from plant treated with *T. harzianum* (10.7%), while the lowest had fruits treated with *B. suptilis* (10.2%). In the first harvest values of ascorbic acids in fruits ranged between 32.6 mg to 35.2 mg. Fruits from control plants had significantly higher values of AA. Generally, fruits from the second harvest had a decreased of contents of chemical properties.

Key words: strawberry, microorganisms, fruits quality, yield

Introduction

The cultivated strawberry (Fragaria × ananassa Duch.) is the most important soft fruit worldwide (Natsheh et al., 2015). The strawberries are economically important owing to their unique flavour of fruits, health benefits, and nutritional aspects (Shen et al., 2019). Also, strawberry is an excellent source of natural antioxidants, such as carotenoids, phenolics, vitamins, anthocyanins and flavonoids, with a remarkably high capacity for scavenging free radicals (Giampieri et al., 2012). Annual production of strawberry is more than 8.1 million tons (FAO, 2022) but, loses during postharvest strawberry storage are estimated to be as high as 40% (Duran et al., 2016).

At last two decades the new agro-techniques have been developed with special emphasis on the use of plant growth-promoting microorganisms (PGPMs). Biofertilizers or biostimulants, containing beneficial microorganisms, possess the ability to aggressively colonize the rhizosphere, plant roots or both when applied to seed or crops. Those microorganisms have shown potential to promote plant growth by the release of metabolites into the rhizosphere which stimulating growth. Their activity is reflected in plant growth through the secretion of plant growth hormones such as auxin, which enhances root hair growth and lateral root development in plant. The improved of root volume increases the rate of nutrient uptake from the soil, which leads to enhanced plant growth and fruit yield and support plant development under natural or stressed conditions, as well as increasing yield and quality of strawberry (Cho and Lee, 2013; Vejan et al., 2016). Moreover, they can improve plant nutrition, increased fruit quality, in terms of taste and nutritional value (Kundan et al., 2015; Morais et al., 2019).

However, biopesticides and biostimulants also have certain disadvantages, including: more difficult implementation, narrower spectrum of action, slower action compared to synthetic pesticides, exclusively preventive action, shorter shelf life, incompatibility with other compounds, and need for frequent application (Khirallah et al, 2016). However, the effect of plant growth-promoting microorganisms on strawberry yield and quality has been poorly studied due to the difficulties associated with field experiments (Chebotar et al., 2022). Nevertheless, we found only a few publications connected to this topic. A combined application of PGPMs strains *Rhizobium* sp., *Bacillus* sp. and *Herbaspirillum* sp. significantly increased the productivity of strawberries and reduced the use of chemicals. Similar results were obtained when using PGPR strains *Pseudomonas* sp (Anuradha et al., 2020; Bayramoglu et al. 2020). The goals of this study were examined an influence of different microorganisms to improve yield and quality of fruits strawberry cultivar ‘Clery’.

Materials and Methods

Plant, research design and environmental measurements

The research was performed in the strawberry open field plantation, which was established in 2018 year on an area of 3 ha, in the village of Selevac, Central Serbia. Plantation was built in the first half of August, by planting cold stored (frigo) plants. A single-row system of cultivation on banks has been applied. The distance of planting hedges in the banks is 16.5 cm, that is, 6 plants were planted per meter of the bank. The orchard was managed according to the integrated cultivation system, on sandy loam soil type, with an average pH of 6.3. The experiment was set up according to a complete block design with four repetitions (30 plants per repetition). During period close to a blooming (first half of September) all of flowers were hand removed. The researche were examined an influence of following microbiological preparations: *Bacillus subtilis* 10^9 **CFU/ml (**colony-forming unit per millilitre) **and** *Trichoderma harzianum* (10^{10} **CFU/ml)**, on productively properties of strawberry plants and physical and chemical quality of fruits. The microbiological preparations were applied by drip irrigation system in doses of 5 l/ha, two and four weeks after planting, and also, next spring four and two weeks from the expected beginning of blooming. Fruit were harvested at commercial maturity stage and transported to our laboratory within 2 h. The fruits were selected based on uniformity of size, shape, and color, and the absence of external physical damage and fungal infection.

Determination of plant phenological properties, productivity and physical properties of fruit

According UPOV Code for strawberry the following phenological properties were studied: beginning of blooming - when 10% of flowers are open, full blooming - when 90% of flowers are open, and beginning of harvest – when 90% of the surface showing red color. The following properties of productivity were analyzed: the number of crowns per plant, diameter of crowns, the number of inflorescences per crowns, the number of fruits per inflorescence, the total number of fruits per plants and the yield per plants (total and separately per harvesting in kg). The examined of physical traits of fruits after harvesting included: mass of fruit (g), a length and width of fruit (mm) and firmness (kg/cm^{-2}).

The firmness of fruits was determined with table digital firmness analyser (AMSTAT with diameter of 4.0 mm). The parameters for firmness analysis were as follows: trigger force = 10 g and penetration depth = 5 mm. Physical properties of fruits were determined with four repetitions, and each repetition included 15 fruits. Analysis of variance has been done with STATISTICA 9 software package. The significant differences between means determined at

P<0.05, measured with Duncan's test.

Determination of total soluble solids (TSS), titratable acidity (TA) and ascorbic acid (AA)
After harvesting, the fruits were stored at 3° C and analysed within 24 h. In total, 500 g of undamaged berries were selected and manually crushed to obtain the juice. For analyses, juices were centrifuged at 14,000× *g* for 20 min. The chemical properties of fruits were determined with four repetitions Total soluble solids (TSS) content of berries was determined using by refractometer (Atago, pocket PAL-1. Kyoto, Japan). Titratable acidity (TA) was determined by titrating the berry juice with 0.1 N NaOH up to pH 7.0. Acidity was expressed as percent of malic acid. Ascorbic acid was quantified using a reflectometer set from Merck Co. (Merck RQflex, Darmstadt, Germany). Fruit samples (5 g) and 20 mL oxalic acid (1%) were mixed, homogenized for 1 min and filtered. Polyvinyl-polypyrrolidone (PVPP) (500 g) was added to 10 mL of the filtered sample to remove phenols, and 6–7 drops of H_2SO_4 (25%) were added, to reduce the pH level below 1.14. Results were expressed as mg ascorbic acid 100 g^{-1} FW.

Results and Discussion

The plant growth-promoting microorganisms had influence to phenological properties of strawberry plant (Figure 2.). The control plants had early beginning of blooming compared to others treatments. Nevertheless, all plants supplied with PGPMs had early harvesting stages compared to control plants.

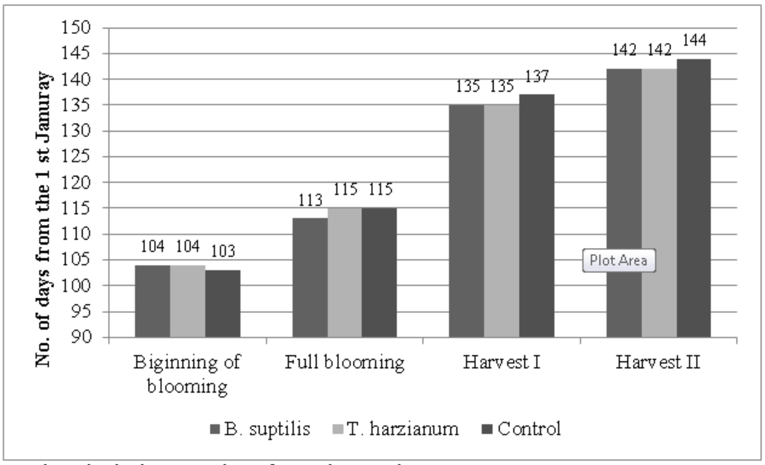

Figure 2. Phenological properties of strawberry plants

The plant growth-promoting microorganisms had significantly influence to strawberry plant productivity (Tab. 1). The number of crowns per plant was between 2.6 and 2.9. Plants supplied with *T. harzianum* and *B. suptilis* had significantly higher diameter of crowns and numbers of inflorescences per crowns compared to control plants. The numbers of flowers per inflorescences was between 7.0 (control plants) to 9.8 (plants treated with *B. suptilis*). The applied a PGPMs improved and enhanced the root system development of strawberry plants which contributed of high production of crowns per plants. Auxins are responsible for the division, extension, and differentiation of plant cells and tissues and are known to increase the rate of xylem and root formation (Chebotar et al., 2022).

Table 1. Productivity properties of strawberry plants treated with PGPMs

Treatment	Number of crowns per plant	Diametre of crowns (mm)	No of inflor. per plant	No of fruits per inflor.	Number of leaves per plants	Yield of 1st harvest (kg)	Harvested fruits I harvest (%)	Yield of 2nd harvest (kg)	Harvested fruits II harvest (%)	Total yield (kg)
B. suptilis	2.8 a	15.5 a	2.9 a	9.8 a	23.3 b	0.18 a	22.2 ab	0.24 b	38.5 b	0.42 b
T. harzianum	2.9 a	17.2 a	3.1 a	9.7 a	29.5 a	0.22 a	25.3 a	0.34 a	46.2 a	0.56 a
Control	2.6 a	13.4 b	2.1 b	7.0 b	24.8 b	0.12 b	19.5 b	0.26 b	40.8 b	0.38 b
lsd	0.5	2.0	0.6	1.6	3.6	0.04	5.6	0.04	3.2	0.08

Data are means of four replications; Different letters in columns indicate significant difference between cultivars according to Duncan's test at 5% level ($p < 0.05$)

The plants treated with *T. harzianum* had the highest yield of fruits in first harvest (0.28 kg), and followed plats treated with *B. suptilis* (0.18 kg). All applied PGPMs contributed to higher yield harvested fruits in first harvest compared to control plants. Further, a percentage of harvested fruits in first harvest in regard to total fruits per plants were between 19.5% (control plants) to 25.3% (*T. harzianum*). In the second harvest, to all treatments were recorded an increased yield of harvested fruits compared to first harvest. Plants treated with T. *harzianum* had the highest yield in second harvest (0.34 kg), while plant treated with *B. suptilis* had the lowest (0.24 kg). Also, percentage of harvested fruits in second harvest was higher compared to first harvest. The highest percentage harvested fruits had plants treated with *T. harzianum*, while the lowest plants treated with *B. suptilis*. A total yield of marketable fruits per plants ranged between 0.38 kg (control) to 0.56 kg (*T. harzianum*). Plants treated with T. *harzianum* had significant higher values of this property compared to other treatments. Also, results of other authors show that plants grown promoting bacteria such as *Bacillus simplex*, *Paenibacillus polymyxa* and *Bacillus* spp**.,** have the potential to increase the yield and growth of strawberries (Erturk et al., 2012). PGPMs specifically activate growth regulators such as auxin, gibberellins, cytokinin, inorganic phosphorus solubilization and nutrient mineralization along with symbiotic N-fixation (Zahir et al., 2004).

In the first harvest the highest mass of fruits had plants treated with *T. harzianum* (34.2 g), while the lowest had control plants (Tab. 2). According to Mikiciuk et al. (2019) applied species of rhizosphere bacteria and mycorrhizal fungi increase the yield and mean weight of strawberry fruit, due to improved activity of roots which contributed uptake of nutrient from soil. Also, enhances root hair growth and lateral root development in strawberry plants had influence increased of yield (Meng et al., 2016). The firmness of fruits harvested from plants treated with T. *harzianum* was significantly higher compared to fruits from control and plants treated with *B. suptilis*. The firmness of fruits in first harvest ranged between 3.4 kg/cm^2 to 4.7 kg/cm^2. In the second harvest the highest mass of fruits had plants treated with *T. harzianum* (22.5 g), while the lowest had control plants. To all treatments in second harvest were recorded higher values of fruits firmness compared with fruits picked in the first harvest. Similar like in the first harvest, a firmness of fruits harvested from plants treated with *T. harzianum* (4.9 kg/cm^2) was significantly higher compared to fruits from control and plants treated with B. suptilis (4.1 kg/cm^2 and 4.3 kg/cm^2, respectively).

Table 2. Physical properties of fruits harvested in the first and second harvest

Treatment	Fruits mass (g)	Fruits lenght (mm)	Fruits width (mm)	Firmness (kg/cm2)	Fruits mass (g)	Fruits lenght (mm)	Fruits width (mm)	Firmness (kg/cm2)
	I harvest				II harvest			
B. suptilis	33.2 ab	52.4 a	41.6 b	3.4 b	21.3 ab	32.5 a	31.2 ab	4.3 b
T. harziaunum	34.2 a	48.5 b	43.2 a	4.7 a	22.5 a	31.2 a	32.5 a	4.9 a
Control	31.1 b	48.2 b	41.2 b	3.4 b	19.2 b	28.4 b	29.6 b	4.1 b
lsd	2.7	3.1	1.4	0.8	3.2	2.7	2.4	0.4

Data are means of four replications; Different letters in columns indicate significant difference between cultivars according to Duncan’s test at 5% level ($p < 0.05$)

The plant growth-promoting microorganisms had significantly influence to fruits chemicals quality in the first harvest (Tab. 3). The highest content of TSS had fruits from plants treated with T. *harzianum* (10.7%), while the lowest had fruits treated with *B. suptilis* (10.2%). In the first harvest values of ascorbic acids in fruits ranged between 32.6 mg to 35.2 mg. Erturk et al. (2012) were noticed that bacteria also increased soluble solid content (SSC) and vitamin C in strawberry. Based on the assessment of the condition of the photosynthetic apparatus and the analysis of chlorophyll "a" fluorescence indices, including hierarchical cluster analysis, the rhizosphere bacteria were found to have favorable effects on strawberry plants which can likened with improved chemical properties of strawberry fruits (Paliwoda et al., 2022). Fruits from control plants had significantly higher values of AA. Generally, fruits from the second harvest had a decreased of contents of chemical properties compared to fruits from the first harvest. The highest decreased of chemicals traits in fruits of second harvest were recorded to values of total acids and ascorbic acids.

Table 3. Chemical properties of fruits harvested in the first and second harvest

Treatment	TSS (%)	TA (%)	AA (mg/100 g f.w.)	TSS (%)	TA (%)	AA (mg/100 g f.w.)
	I harvest			II harvest		
B. suptilis	10.2 b	0.84 a	35.6 a	10.8 a	0.81 a	31.6 b
T. harzianum	10.7 a	0.76 a	33.4 b	10.7 a	0.47 b	30.5 b
Control	10.5 ab	0.86 a	34.2 b	10.4 ab	0.72 ab	33.6 a
lsd	0.4	0.18	1.1	0.3	0.32	1.5

Data are means of three replications; Different letters in columns indicate significant difference between cultivars according to Duncan's test at 5% level ($p < 0.05$)

Conclusion

According to ours results used plant growth-promoting microorganisms had significantly influence to plants productivity and fruits quality of strawberry cultivar 'Clery'. A plants supplied with *T. harzianum* had significantly higher values of examined productivity properties, especially when it comes to yield in second harvest and total yield of marketable fruits. Further, plants supplied with *B. suptilis* had significantly higher values of chemicals properties of fruits. Also, applied PGPMs had influences to improve physical properties of fruits. Due to improved of productivity properties and increased of fruits quality apply of plant grown promoting microorganisms it should be part of practice in strawberry growing.

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Comparative analysis of potential clones of the Žilavka variety in region of Herzegovina, subregions of the middle Neretva and Trebišnjica

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Abstract

In the paper, isolated clones of the autochthonous variety Žilavka in the region of Herzegovina, subregions of the middle Neretva and Trebišnjica in the vineyard of the monastery Tvrdoš were examined. Phenological characteristics, fertility, mechanical composition of grapes and berries and chemical quality were studied. The laboratory tests were carried out in the laboratory of the Department of Viticulture of the Faculty of Agriculture University of Belgrade. The coefficient of absolute fertility had the highest values in clone 10 (2.0) and the lowest in clone 9 (1.3). According to the values of the coefficient of absolute, relative and potential fertility, clone 7 stood out. Clone 8 was characterised by the largest mass of grapes (329,2 g) and berries (315,4 g), as well as one of the largest mass of 100 seeds (3,0 g). The most sugar in grape juice was accumulated by clone 10 (22.7%), and the least by clone 7 (16.2%) and the population (16.5%). Clone 3 was characterised by high content of total acids (5.7 g/l), while clones 5 and 8 had very low content.

Key words: Žilavka, clone, fertility, mechanical composition of grapes, grape juice

Introduction

According to the International Organization of Vine and Wine (OIV), 7,327,174 ha are under vine worldwide in 2019. The largest areas are in Europe (3,702,167 ha), followed by Asia (2,055,379 ha), North and South America (1,032,165 ha), Africa (366,755 ha), while the smallest areas are in Australia (185,305 ha). Among the countries (Khan et al., 2020), the largest areas are in Spain (966 442 ha) and France (794 336 ha), followed by China (780 665 ha), Italy (713 146 ha) and Turkey (435 846 ha). In Bosnia and Herzegovina, according to the Agency for Statistics of Bosnia and Herzegovina for the year 2021, 2873 ha were planted with vines, of which 541 ha were in the Republic of Srpska.

The origin and development of viticulture in Herzegovina dates back to the time of the Greeks and Romans, more than two thousand years ago, and continued in the Middle Ages, mainly thanks to Christianity. From that time there are preserved documents mentioning viticulture and wine, and the most significant is certainly the document of King Tvrtko I Kotromanić from 1353. With the advance of the Ottoman Empire, the stagnation of viticulture and wine production in Herzegovina began. During this period, the monasteries were the only places where viticulture was practiced.

With the occupation of Bosnia and Herzegovina by Austria-Hungary in 1878, viticulture experienced a boom. Fruit and wine growing stations were established in Derventa, Mostar and Lastva near Trebinje, and with them new ampelotechnical measures were introduced, such as deep plowing, selection of varieties, use of scissors as pruning tools, setting stakes along the vines and tying them up, and in wine production began regular use of the process of clarification, filtration, bottling, etc. To train the population, whole families of winemakers migrate from Hungary (Beljo et al., 2018). In addition to European varieties, special attention is paid to the two autochthonous Herzegovinian varieties, Blatina and Žilavka. Then, the first descriptions of the main Herzegovinian wine varieties Žilavka, Blatina, Krkošija, Bena, Trnjanak, Dobrogostina, Pošip white and black and Rezakija were prepared. It is believed that Herzegovinian wines from Lastva and Mostar reached the table of the Viennese court, which is why these vineyards were called "imperial". According to Mijatović (1988) and Blesić et al. (2013), the Austro-Hungarians used Žilavka for the production of special wines thanks to the quality of grapes and resistance to gray mold (Botrytis cinerea Perc.). Grape production was increased until the end of the 19th century, when late blight decimated the vineyards of Herzegovina. The appearance of phylloxera in Herzegovina in 1912 and the First World War led to the decline of many vineyards. After the end of the war, the orchards and vineyards stopped working and the renovation of the vineyards began. In the period between the two wars, there was no significant renewal of viticulture.

After the Second World War began the revival of viticulture based on plantation cultivation, especially with the variety Žilavka. Žilavka is an autochthonous Herzegovinian variety, which, according to Žunić et al. (2017) and Vukojević et al. (2022), has been cultivated in Herzegovina for more than 600 years and was first mentioned in the 14th century. It is believed to get its name from the veins visible through the skin when fully mature, while others claim that the "tough" character of the vine, which withstands difficult conditions in the vineyard, is responsible for its name Memic et al. (2012). Its synonyms are Mostar Žilavka, Žilavka bijela, Žilavka Herzegovačka, belongs to Proles pontica (convarietas pontica). Autochthonous varieties have got a very important position in modern grape production. Clonal selection is appropriate tool to select plants with better morphological and technological properties from a plants population which is characterized with high variability. According to Marković et al., (2017) for clonal selection the most important parameters are: vigor, shape and bunch size, bunch and ampelographic composition of berry, grape and wine quality as well as disease resistance ect. The results presented in this paper are one of the first studies related to the clonal selection of the Žilavka variety in wine subregions of the middle Neretva and Trebišnjica.

Material and Methods

In the work, the economic and technological characteristics of various clones of the autochthonous variety Žilavka were studied. The experiment was carried out in the vineyard of the Tvrdoš monastery, founded in 1948, with a planting distance of 1.8 m between rows and 1 m in the row, with the rows oriented in the north-south direction. The exemplary vineyard is in Lastva, near Trebinje, on the hill Brežine, 400 m above sea level. The production vineyard belongs to the region of Herzegovina, to the sub-region of Middle Neretva and Trebišnjica, and to the vineyard of Mostar. The tests included eleven potential clones of the Žilavka variety. In experimental vineyard it is present bilateral symmetrical cordon on which short pruning was used on each of the clones. The selection of clones was made by vigor, shape and size of bunches, ampelographic composition of berries and quality of grape juice. Laboratory tests were performed in the laboratory of the Department of Viticulture, Faculty of Agriculture University of Belgrade. Research included examination of phenological characteristics of clones (Lorenz et al., 1994), fertility, mechanical composition of clusters and berries, and chemical quality of grape juice. Fertility is shown through coefficients of potential, relative and absolute fertility. Grapes were harvested at full maturity (phenolic maturity). Mechanical analysis of grape (bunches) and berries was done by Marković (2012) and Marković and Przić (2020) methodologies. Bunches (10 per clone) were measured for their weight, length, and width. Number of berries per bunch was also determined and berry mass per bunch and mass of stems were measured on analytical balance. From each variety, 100 representative berries were selected, and after measuring the mass of the berries, the berry skin and seeds were separated. The mass of seeds and skin of 100 berries was measured on an analytical balance. Among the chemical parameters of grape juice composition (must), the following were determined: sugar content (%), total acid content (g/l) and pH value. Sugar content was determined by physicochemical methods using Oeshle mostwage, values were calculated using Dujardin-Salleron tables. The total acid content was determined by titration with n/4 NaOH and pH using a pH-meter. The glycoacidometric index was calculated from the ratio of sugar and acid content. Statistical analysis of data was performed using multifactorial analysis of variance (ANOVA).

Results and Discussion

Phenological observations revealed the beginning of bleeding, which started on April 25 and lasted 14 days. Flowering began on June 16 and lasted 12 days. Veraison occurred on average on August 17, and full maturity around October 3. From bleeding to full maturity was 162 days. There was no significant difference between clones in the duration of phenophases.

Table 1. Phenological observations

<table>
<tr><th>Phenophases</th><th>Start</th><th>End</th><th>Duration (days)</th></tr>
<tr><td>Bleeding (Cod 00-03)</td><td>25.04</td><td>08.05</td><td>14</td></tr>
<tr><td>Flowering (Cod 60-69)</td><td>15.06</td><td>26.06</td><td>12</td></tr>
<tr><td rowspan="2">Maturity</td><td>Veraison (Cod 81-83)</td><td>17.08</td><td rowspan="2">48</td></tr>
<tr><td>Grape harvest (Cod 85-89)</td><td>3.10</td></tr>
<tr><td colspan="3">Number of days from bleeding to harvest</td><td>162</td></tr>
</table>

The coefficient of absolute fertility varied from the lowest (clone 9) to high values (clones 5, 7, 8, and 10) depending on the clone. The highest value of this indicator was in clone 10 (2.00), and the lowest in clone 9 (1.36). The absolute fertility rate of the population was 1.78. All clones except clone 3 had a very high coefficient of relative fertility. The lowest value of this coefficient was in clone 3 (1.17), and the highest in clones 7 (1.92), 5 (1.91) and 8 (1.91). The relative fertility rate of the population was 1.45, and the coefficient of potential fertility varied from 1.17 (clone 3) to 1.92 (clone 7). The results are presented in Table 2.

Table 2. Coefficients of absolute, relative and potential fertility

Clone	Coefficient of absolute fertility	Coefficient of relative fertility	Coefficient of potential fertility
1	1.63	1.50	1.50
2	1.67	1.70	1.21
3	1.70	1.17	1.17
4	1.75	1.75	1.75
5	1.91	1.91	1.75
6	1.82	1.67	1.67
7	1.92	1.92	1.92
8	1.91	1.91	1.75
9	1.36	1.25	1.25
10	2.00	1.82	1.67
Population	1.78	1.45	1.33

The average weight of the cluster varied from 161.0 g (clone 6) to 329.2 g (clone 8). Similar results were obtained by Jovanović-Cvetković et al. (2016) in the examination of Žilavka in the locality of Mostar (170.06-325.45 g), and this results do not differ significantly compared to the locality of Trebinje. Most clones were characterized by higher average mass compared to the population (clones 1, 3, 4, 7, 8 and 9), which was statistically proven. The largest variation were found between clones 6 and 8, and 8 and 10.

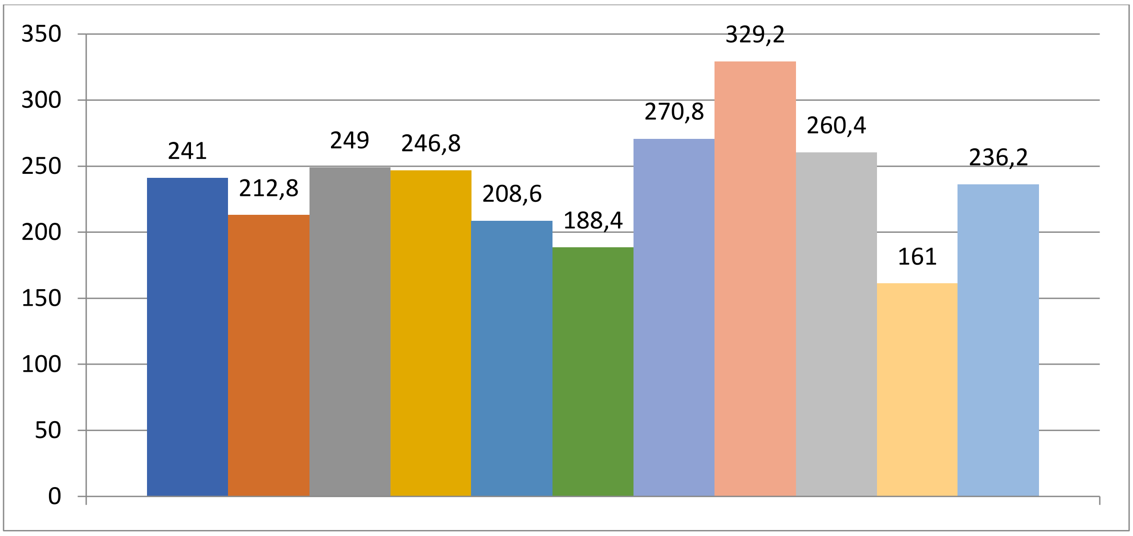

Figure 1: Average mass of bunches of clones of the Žilavka variety (g)-from left to right clone 1,2,3,4,5,6,7,8,9,10 an population

Table 3: ANOVA results for average cluster mass (g)

Source of variation	Sum of squares (SS)	Number of degrees of freedom	Mean Sum of Squares (MS)	F statistic	*p*-value
Between groups	99.884,84	10	9.988,48	2.5656	0.015
Inside groups	171.299,60	44	3.893,17		

A statistically significant variation was found in the average mass of berries between clones and between clones and the population. Clones 10, 6, 5 and 2 had lower average berry weight compared to the population, while the highest value of this parameter was obtained for clones 8 (315.4 g) and 10 (154.0 g). The results are presented in graph no. 2 and table no. 4. Similar results were obtained by Markovic et al. (2017) and Pržić & Markovic (2019) examining clones of Prokupac variety.

Table 4. ANOVA results for the average weight of all berries in a cluster (g)

Source of variation	Sum of squares (SS)	Number of degrees of freedom	Mean Sum of Squares (MS)	F statistic	*p*-value
Between groups	92.484,00	10	9.248,40	2.42	0.02
Inside groups	167.572,00	44	3.808,45		

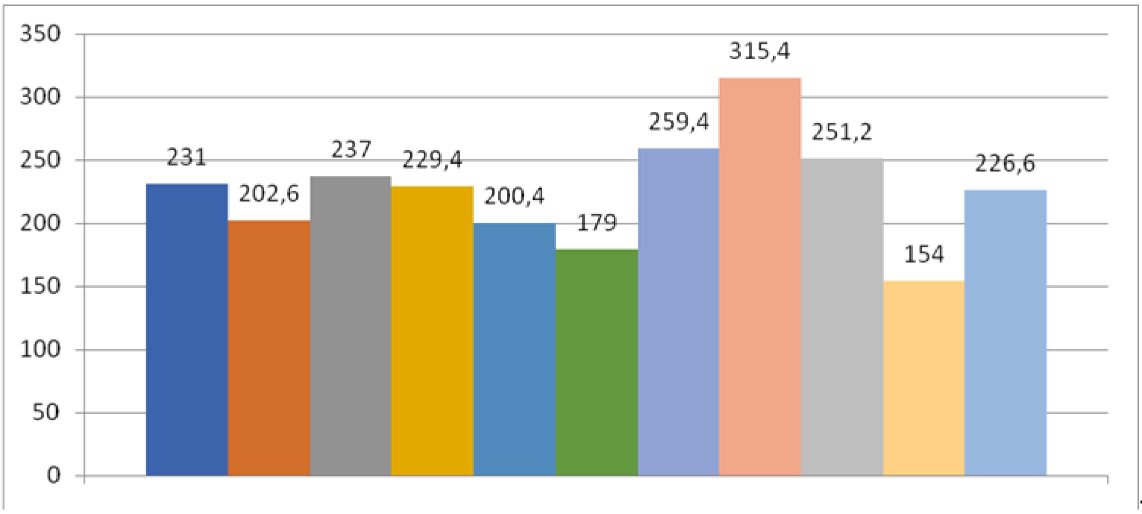

Figure 2. Average mass of all berries in a bunch (g)-from left to right clone 1,2,3,4,5,6,7,8,9,10 an population

The analysis of the mechanical parameters of the berries (Table No. 5) included the following parameters: weight of 100 berries (g), weight of skin of 100 berries (g), weight of seeds of 100 berries (g), number of seeds in 100 berries and weight of 100 seeds (g). The weight of 100 berries varied from 169 g in clone 10 to 295 g in clone 7. Clone 10, with a weight of 100 berries of 169 g, is the only clone that showed a lower value compared to the population (215 g). When analyzing bery skin weight 100 berries, the lowest value was obtained for the population (8.55 g) and the highest for clone 3 (16.9 g). The lowest value of the mass of seeds in 100 berries was determined for clone 10 (3.44 g), the highest for clone 1 (7.14 g), while for the population was 5.87 g. The number of seeds in 100 berries varied from 104 (clone 6) to 223 (clone 1).

Table 5: The values of the analysis of the mechanical composition of the berries

Parameter/clone	1	2	3	4	5	6	7	8	9	10	P
Mass of 100 berries	265	237	240	288	234	248	295	248	228	169	215
Bery skin weight 100 berries	11.3	12.2	16.9	11.2	9.1	10.2	10.0	9.2	9.5	9.1	8.5
Seed mass of 100 berries	7.1	5.7	6.4	6.0	6.2	4.4	5.5	6.0	5.9	3.4	5.8
Number of seeds 100 berries	223	167	187	180	183	104	155	181	186	121	186
Mass of 100 seeds	3.4	3.0	3.2	2.9	2.9	3.7	3.0	3.0	2.9	2.3	2.8

P-population

The sugar content of grape juice varied from 16.2% (clone 7) to 22.7% (clone 10). Besides clone 7, low accumulated sugar content was determined for the population (16.58%). The highest acidity was found in clone 3 (5.7 g/l), while clones 5 and 8 had the lowest values (4.50 g/l), while the population accumulated 5.10 g/l. In the studied clones, pH varied from 3.09 (clone 9) to 3.23 (clone 1 and clone 7). This results was according to Žunić and Garić (2017).

Table 6. Chemical composition of the grape juice clones of the Žilavka variety

	1	2	3	4	5	6	7	8	9	10	P
Sugar (%)	19.6	22.3	22.0	16.9	19.6	21.5	16.2	18.8	19.1	22.7	16.5
Acids (g/l)	5.4	5.1	5.7	4.7	4.5	5.4	4.6	4.5	5.2	4.4	5.1
pH	3.23	3.13	3.21	3.15	3.21	3.18	3.23	3.16	3.09	3.11	3.10
Gly	3.58	4.39	3.87	3.58	4.36	3.93	3.48	4.19	3.64	4.14	3.25

P-population; Gly- Glycoacidometric index

Conclusion

Based on results the following conclusions can be made. The highest number of fertile shoots was observed in clones 4 and 7. Clone 3 showed the lowest number of developed shoots. The coefficient of absolute fertility had the highest value for 10 and the lowest for clone 9. The coefficients of relative and potential fertility were highest for clone 7. Parameters of mechanical analysis of the cluster, such as weight, number of berries in cluster, proportion of seeds and pulp in berry, had higher values relative to population. Clone 8 had the highest cluster mass and the average largest mass of all berries per cluster. From the analysis of qualitative parameters (sugar and total acidity), it can be concluded that clone 10 had the highest sugar content and clone 7 and the population had the lowest. Clone 3 had the highest and 10 had the lowest content of total acids. For most of the tested parameters, most clones were better than the population. On the basis of technological features, clones 2, 3, 8 and 10 are recommended.

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Influence of microbiological preparation Bacillomix on the germination of *Challistephus chinensis* L.

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Abstract

The use of biostimulant create better conditions for the growth and development of the germ and germination. Bacillomix is 100% natural biostimulant and soil improver with increased content of live bacterial cells obtained from 12 powerful Bacillus sp. strains. The use of this preparation has many positive effects: stimulate of germination, rooting and growth of plants; improve the microbiological structure of the soil; keep the plants healthy through the growing season; increases the plants resistant to stress; supplies the plant with nitrogen, phosphorus, potassium and sulfur. The aim of this study was to examine the impact of microbiological preparation Bacillomix on the germination and germination energy of the seeds *Challistephus chinensis* L. In the experiment were totally 480 seeds of this species. The experiment was set up in the laboratory on Faculty of Agriculture, University of Banja Luka and consisted of control (K) and treatment with microbiological preparation Bacillomix in two concentrations (T1 100% and T2 10%). After 7 days germination energy and after 14 days germination of the seeds were tested. The highest average values of the germination energy were recorded in the treatment plants (73.75% T1, 64.38% T2), while the lowest values were recorded in control plants (60%). The highest average germination values were also recorded in treatment (78.755% T1, 73.75% T2), and the lowest in the control group (70%). The highest average values of hypocotyl height, root length and fresh weight were recorded also in the treatment plants (1.47 cm T1 and 1.01 cm T2; 4.59 cm T1 and 3.28 cm T2; 0.91 g T1 and 0.65 g T2) while the lowest values were recorded in control plants (0.99 cm; 2.87 cm; 0.61 g). It can be concluded that the use of a natural biostimulant is recommended for better germination and better morphological development of plants

Key words: Bacillomix, germination, flower

Introduction

Successful floriculture production requiers knowledge of plant morphology and of all other factor on their growth and development. Aster chinensis - *Challistephus chinensis* L. is an annual flower and one of the most used summer ornamental plants in the gardens whose blossoming extending from early summer to late autumn. This flower belongs to the Asteraceae family and thanks to wide variety of plant hights, forms, and flower size and colors is a very popular among gardeners. Aster chinensis can be used as potted flowers (dwarf varieties) or as cut flowers (tall varieties). It can be easily propagated by seed with minimal labor requirements. For the propagation of annual flower seedling or for direct sowing, seed quality is essential. Poor and ununiformed germination is an undesirable seed property. Fast and homogenous germination provide both economic and environmental benefits in agriculture and horticulture (Badek et al. 2006). The germination process is characterized by increase in the activity of hormones and enzymes in the seed during the water absorption. By applying biostimulant in the germination process, it is possible to create better conditions for the growth and development of the germ and germination. The seed that has better germination energy also has better vigor, so it is more resistant to stressful conditions during germination. Biostimulants can be applied in various stages - from sowing seed to transplanting and after transplanting in gardens (Vernieri i sar. 2002; Parađiković i sar. 2008). Biostimulant application can positively influence seed germination and vigour of older seeds, as it is proved in maize and soya bean (Vinković et al. 2007), celery, parsley, lettuce and leek (Yildirim et al. 2002). It is well known that plants during the process of transplantation go through certain abiotic stress as a result of deviation in the environmental conditions from the optimum (Mena - Petite et al. 2006; Kijne et al. 2006). Plants treated with biostimulants increasingly develop their organs such as roots, stems and leaves, which is a basic postulate for better plant adaptation after transplanting. Bacillomix® is 100% natural biostimulant and soil improver with increased content of live bacterial cells obtained from 12 powerful *Bacillus* sp. strains. The use of this preparation has many positive effects: stimulate of germination, rooting and growth of plants; improve the microbiological structure of the soil; keep the plants healthy through the growing season; increases the plants resistant to stress; supplies the plant with nitrogen, phosphorus, potassium and sulfur.

The aim of this study was to examine the impact of microbiological preparation Bacillomix® on the germination and germination energy of the seeds *Challistephus chinensis* L.

Material and Methods

Investigation was carried out in the laboratory condition at the Faculty of Agriculture, University of Banja Luka, Republic of Srpska/Bosnia and Herzegovina. Flower seeds of Aster chinensis - *Challistephus chinensis* L. were collected from the natural population in Podrašnica village. In additional, microbilogical prepation Bacillomix®, which contains live bacterial cells obtained from 12 powerful *Bacillus* sp. was used.

Experiment was set up in Petri dishes sterilized with 96% ethanol and lined with filter paper consisted of control (K) with distilled water and treatment with microbiological preparation Bacillomix® in two concentrations (T1 100% and T2 10%). Experiment was set-up in four repetitions for each treatment. Fourty seeds were counted and placed in each Petri dishes on the wet paper. For control group the filter paper was sprayed with 5 mL of distilled water and for treatment with 5 mL 100% or 10% of biostimulant Bacillomix®. Prepared Petri dishes with seeds were placed in the growth chamber under an artificial white light for 16h a day and 8h per night. Temperature during the research was constant (20±1°C). Petri dishes were observed daily and additional water or biostimulant was added if needed. Seeds were kept under these conditions for 14 days. After 7 days germination energy and after 14 days germination of the seeds were tested. Both values are expressed as percentage (%). In additional, after 14 days hypocotyl height, root length, and fresh weight were obtained. The obtained data were statistically analysed (LSD, F-test, t-test) using standard computer programs and VV-Stat (Vukadinović, 2017). Means comparison were performed using low significant differences procedure (LSD), with a significance level of 5% ($P<0.05$).

Results and Discussion

Statistically analyzed obtained results of germination energy, seed germination, and number of cotyledons, hypocotyl height, root length, and fresh weight of Aster chinensis - *Challistephus chinensis* L. under influence of microbiological preparation Bacillomix® are present in Table 1. and Table 2.

Treatment T1 – 100% of microbiological prepation Bacillomix®, had the best result in all of three parameters. Germination energy was under very significant influence (p=0.01), while germination rate and number of cotyledons were under significant influence (p=0.05) of Bacillomix®.

The highest average values of germination energy of the Aster chinensis was recorded in the treatment T1 (73.75%), while the lowest value was recorded in the control group (60%). Also, the highest average value of germination rate was recorded in the treatment T1 (78.75%), while the lowest value was recorded in the control group (70%). The highest average developed of hypocotyls were recorded in the treatment T1 (31.50) and the lowest in the control group (27.5).

Table 1. Influence of microbiological preparation Bacillomix® on germination energy, seed germination, and number of cotyledons of Aster chinensis - *Challistephus chinensis* L. seeds (K - control – distilled water; T1 - 100% of microbiological prepation Bacillomix®; T2 - 10% of microbiological prepation Bacillomix®); means marked with different superscript letters are significantly different at p=0.05

Treatment variant	germination energy %	germination rate %	number of cotyledons
Control K	60.00^{c}	70.00^{b}	27.50^{b}
Treatment T1	73.75^{a}	78.75^{a}	31.50^{a}
Treatment T2	64.38^{b}	73.75^{b}	29.50^{b}
Average	66.04	74.17	13.38
Analyses of variance - F	9.554654^{**}	5.549906^{*}	5.333333^{*}
LSD	germination energy %	germination rate %	number of cotyledons
0.05	7.2701	5.9609	2.7704
0.01	10.4455	ns	ns

ns=not significant

Data shown in Table 2. indicates very significant difference (p=0.01) between the average values of hypocotyl height, root length, and fresh weight of Aster chinensis seeds under influence of microbiological preparation Bacillomix®. Treatment T1 had the best result on all of three parameters. The highest average values of hypocotyl height and root length of Aster chinensis were in the treatment T1 (1.47 cm; 4.59 cm), while the lowest average values of hypocotyl height and root length of Aster chinensis were in the control group (0.99 cm; 2.87 cm). The same ratio was in fresh weight with the highest average values in the treatment T1 (0.91 g), and the lowest average values in the control group (0.61 g) (Table 2).

Table 2. Influence of microbiological preparation Bacillomix® on hypocotyl height, root length, and fresh weight of Aster chinensis - *Challistephus chinensis* L. K - control – distilled water; T1 - 100% of microbiological prepation Bacillomix®; T2 - 10% of microbiological prepation Bacillomix®); means marked with different superscript letters are significantly different at p=0.05

Treatment variant	hypocotyl height (cm)	root length (cm)	fresh weight (g)
Control K	0.99^{b}	2.87^{c}	0.61^{b}
Treatment T1	1.47^{a}	4.59^{a}	0.91^{a}
Treatment T2	1.01^{b}	3.28^{b}	0.65^{b}
Average	1.16	3.58	0.72
Analyses of variance - F	34.85508^{**}	14.33047^{**}	8.641614^{**}
LSD	hypocotyl height (cm)	root length (cm)	fresh weight (g)
0.05	0.1460	0.7604	0.1800
0.01	0.2098	1.0925	0.2586

ns=not significant

Compounds containing natural bioactive materials, such as humic and fulvic acids, have received increased attention in recent years (Calvo et al. 2014; Shah et al. 2018) and represent an environmentally friendly tool for increasing crop yield and productivity (González-González et al. 2020). Plant biostimulants include formulations containing a range of substances, compounds and growth-promoting microorganisms, such as those derived from arbuscular mycorrhizal fungi, seaweed extracts or live bacteria cells, are use to regulate or enhance physiological processes in plants.

Solutions of biostimulants can be applied at different plant growth stages, from germination by means of a seed treatment through to the full plant and fruit/flowers at commercial maturity via foliar application or irrigation of the vegetation, and can be added as a supplement to growth media in tissue culture (Parađiković et al. 2019). Parađiković et al. (2008) confirmed that biostimulant Radifarm® increased the germination energy, seed germination and seedling fresh weight of *Portulaca grandiflora* Hook., *Helichrysum bracteatum* Vent., *Tagetes erecta* L. and *Zinnia elegans* L. Additionally, Zeljković et al. (2019) found that the application of biostimulant Radifarm® enhanced the seed vigor and plant productivity of *Bellis perennis* L. and *Viola* x *wittrockiana* Gams.

In the research of Majkowska-Gadomska et al. (2017), ornamental plant species (*Callistephus chinensis, Salvia splendens, Zinnia elegans,* and *Tagetes patula*) responded differently to tested biostimulants used for seed preconditioning. Zinnia seeds treated with this biostimulant had the highest germination energy. Jevđović et al. (2012) investigated influence of biostimulants Epin Ekstra and Cirkon on yield and quality of *Linum usitatisimum* L. and concluded that the applied biostimulant can improve yield of flax (*Linum usitatisimum* L.) as well as germination energy and seed germination.

We can find a lot of published paper regarding uses of different biostimulants at different stages of plants, but there is none related to this microbiological preparation Bacillomix®.

Conclusion

The application of microbiological preparation Bacillomix® for seed preconditioning increased germination energy, germination rate, and all of morphological investigated parameters (hypocotyl height, root length, and fresh weight) of Aster chinensis - *Challistephus chinensis* L. seeds. An even the lower concentration of microbiological preparation Bacillomix® in amount of 10% showed the better average results then the control group of plants, which treated only with distilled water.

From results of this research, the following conclusions are obtain:

- the highest germination rate was in the treatment T1 with 100% of microbiological preparation Bacillomix®, with an increase of 7% over the treatment T2 with 10% of microbiological preparation Bacillomix®; and 13% over the control group of seeds;
- the highest value for the average hypocotyl height was in the treatment T1 with 100% of microbiological preparation Bacillomix®with an increase of 45% over the treatment T2 - with 10% of microbiological preparation Bacillomix®; and 48% over the control group of seeds;
- the highest value for the average root length in the treatment T1 with an increase of 40% over the treatment T2 - with 10% of microbiological preparation Bacillomix®; and 48% over the control group of seeds;

Based on the obtained results we can conclude that the treatment with microbiological preparation Bacillomix®can enhance seed vigor and plant productivity of Aster chinensis - *Challistephus chinensis* L. Application of this nautural biostimulant can ensure the production of high-quality seedlings by increasing the resilience of plants to temperature stress and improving the uptake of nutrients after transplanting in gardens.

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Seed weight and optimal imbibition period for some herbaceous peony (*Paeonia* spp.) species native to Serbia

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Abstract

Herbaceous peonies are plant species with high ornamental and medicinal value. Due to their endangerment, wild peonies are protected by law in Serbia. Possible ways to protect them are propagation and/or cultivation. Peonies can be propagated vegetatively (by rhizome division) or generatively (with seeds). As peony seeds have a double dormancy and their germination is a long-term process, it is important to determine which seed characteristics and pre-treatments have a positive effect on germination. Since peony seed weight is an important seed characteristic, and imbibition period is an effective pre-treatment, the aim of this study was to evaluate the peony species native to Serbia, and to determine seed mass, seed imbibition capacity, the influence of seed weight on imbibition and the influence of habitat on the studied parameters, as the mentioned parameters have hardly been studied for the tested species. The research was conducted in 2021. on three peony species native to Serbia (*Paeonia tenuifolia* L.-fern leaf peony or steppe peony; *Paeonia peregrina* Mill.-Balkan peony or Kosovo peony and *Paeonia daurica* Andrews). According to our one-year research, there was no statistically significant difference in seed weight depending on the natural habitat within the species and also not between *P. peregrina* and *P. daurica*. A significant difference was found in seed weight between steppe peonies and the other two studied species. In agreement with our results, the optimal imbibition time is two to three days for *P. peregrina* and one to two days for *P. tenuifolia* and *P. daurica*. The findings can be used as preliminary research for future peony germination studies.

Key words: Paeonia peregrina Mill, *Paeonia tenuifolia* L., *Paeonia daurica* Andrews, seed mass, soaking time

Introduction

Herbaceous peonies have the greatest plant history of any flowering plant genus, with the significant ornamental value of the flower (Sun et al., 2022), as well as the medicinal value of the root (He et al., 2011), petals (Weixing, 2017; Batinić et al., 2022; Čutović et al., 2022) and seeds (Ning et al., 2015). Seeds have also received much attention recently for their ecological (Rudaya et al., 2021) and edible (Qi et al., 2020) value, as well. Herbaceous peonies are native to the Northern Hemisphere and have adapted to a temperate climate with relatively cold winters and dry and hot summers (Marković et al., 2022). Despite their longevity, they are threatened with extinction in their native habitat due to loss of territory and/or excessive collecting. Although peonies are threatened, the genetic variability of peony seeds is an important source of genetic diversity for the maintenance and regeneration of these populations. In addition, peony seeds can be used to establish new populations of peony species in areas where they are locally extinct.

Peony seeds mature slowly, ripening in late summer and dispersing in autumn (Zhang et al., 2018). At the end of development (Figure 1), the seeds of herbaceous peonies are large and dark (Nanjidsuren et al., 2016). The size and weight of the seeds can be influenced by the location, the position of the plant within the site and the time of harvest (Marković et al., 2022). The seed harvest period ranges from July to the end of October, depending on the species, location (altitude, shade, etc.) and year; *P. tenuifolia* matures earlier in July, while *P. peregrina, P. banatica*, *P. mascula* and *P. officinalis* mature later in August (Marković et al., 2022). The best time to collect seeds to achieve the best possible germination is when the follicle opens and the seed coat darkens (Yu et al., 2007; Zhang et al., 2018). Since peony seeds have a double dormancy and their germination is a long-term process, the determination of seed characteristics and pre-treatments that have a positive effect on seed germination is of particular importance for the conservation of the tested endangered species.

Figure 1. The colour changes during the peony seed maturation

The aim of this study was to determine seed weight, seed soaking capacity, the influence of seed weight on imbibition, as well as the influence of native peony habitat on the studied traits, as these parameters have rarely been studied for the mentioned species due to a limited understanding of the physiology of herbaceous peonies.

Materials and Methods

Seed collection

Peony seeds were collected at full maturity from their native habitats and from the Institute's collection. *P. tenuifolia* was collected in July 2021, followed by *P. daurica* and *P. peregrina* in August of the same year. *P. tenuifolia* was harvested from two natural habitats (Deliblato sands, and Gulenovci), *P. daurica* from one natural habitat (Korube) and *P. peregrina* from four natural habitats (Bogovo guvno, Pirot, Golina and Krivi vir) and from the collection of the Institute in Pančevo. The *P. peregrina* populations at the above-mentioned localities, as well as the *P. tenuifolia* populations at Deliblato Sands, each had more than 100 plants; therefore, one third of the seeds of about 30 plants were taken, giving a total number of about 300. The populations of *P. tenuifolia* at locality Gulenovci and of *P. daurica* at Korube are smaller, so about 100 seeds were collected for each locality in order not to endanger the populations. The Ministry of Environmental Protection of the Republic of Serbia has granted permission for wild-collecting (No. 353-01-1467/2021-04, issued on 26. May 2021).

Table 1. Altitude, latitude, and longitude of Serbian herbaceous peony species' habitats (natural and institute collection)

Locality	Altitude (m a.s.l)	Latitude	Longitude
Pancevo-collection	74	44°52'N	20°42’E
Deliblato sands	167	44°57'N	21°03’E
Golina	299	43°46’N	22°19’E
Krivi vir	467	43°49'N	21°46’E
Pirot	666	43°07'N	22°27’E
Gulenovci	840	43°06'N	22°49’E
Bogovo guvno	952	43°33'N	21°46’E
Korube	954	43°30'N	21°42’E

Determination of the dry weight and moisture content of seeds

After seed collection, the dry weight and moisture content of the seeds were determined, followed by measurements of imbibition and seed weight. The measurement of seed moisture content was carried out in a drying oven. Ten seeds from each locality were dried in an oven at 105 °C until no change in weight could be detected. Therefore, measurement was conducted before drying and then after 24, 36 and 48 hours in the oven. The following formula was used to calculate the moisture content of the seeds:

$$\text{\% Moisture content} = \frac{\text{Weight of fresh seeds} - \text{Weight of dry seeds}}{\text{Weight of fresh seeds}} \text{x}100$$

Seed weight measuring

At the beginning of the experiment, undeveloped seeds were isolated and calculated separately; their percentage ranged between 3% and 6% (Table 1). Normal seeds were divided in triplicate before the mass of each seed per location was weighed on an electronic balance. The mean mass of the seeds per site, as well as the variation from the mean value, were calculated using the collected data. The differences between localities were also analysed. The mass of seeds was recalculated based on the mass of absolutely dry seeds.

Imbibition capacity

To investigate the impact of a seed imbibition period (control, 1 to 7 days) at a temperature of 22 ^{o}C under laboratory conditions, 30 seeds of each investigated species from each locality, as well as 30 seeds from the Institute collection, were examined. To establish the initial weight, the mass of the seeds was measured before soaking and at 24-hour intervals. In three repetitions, the seeds were kept separately at room temperature in distilled water, wiped with dry blotting paper, and weighed to validate the maximum soaking capacity. The imbibition rate was calculated using the following formula:

$$\text{Imbibition rate} = \frac{\text{Imbibed weight} - \text{Initial weight}}{\text{Imbibed weight}} \text{x}100$$

Results and Discussion

Seed moisture

Based on the results of seeds collected in 2021, the moisture content of peony seeds ranged from 8.3 to 14.5%, depending on locality and species. No difference was observed between the replicates. (Table 2). The difference in moisture level was noticeable within the species of *P. peregrina* but was not significant, most likely the crucial factor for the difference was in altitude of the native habitats of mentioned species. There was a significant difference between the species. Although steppe peonies have smaller seeds and grow in drier environments than other tested peony species, their seeds probably have a higher water-holding capacity.

Table 2. Average seed moisture content of tested herbaceous peony species based on locality

Species	Locality	Average moisture content (%)	STDV
P. peregrina	Bogovo guvno	10.24	0.10
	Pirot	9.71	0.25
	Pančevo	9.64	0.22
	Krivi vir	9.37	0.26
	Golina	8.64	0.21
P. tenuifolia	Gulenovci	14.03	0.14
	Deliblato sand	13.85	0.23
P. daurica	Korube	14.54	0.18

Seed weight

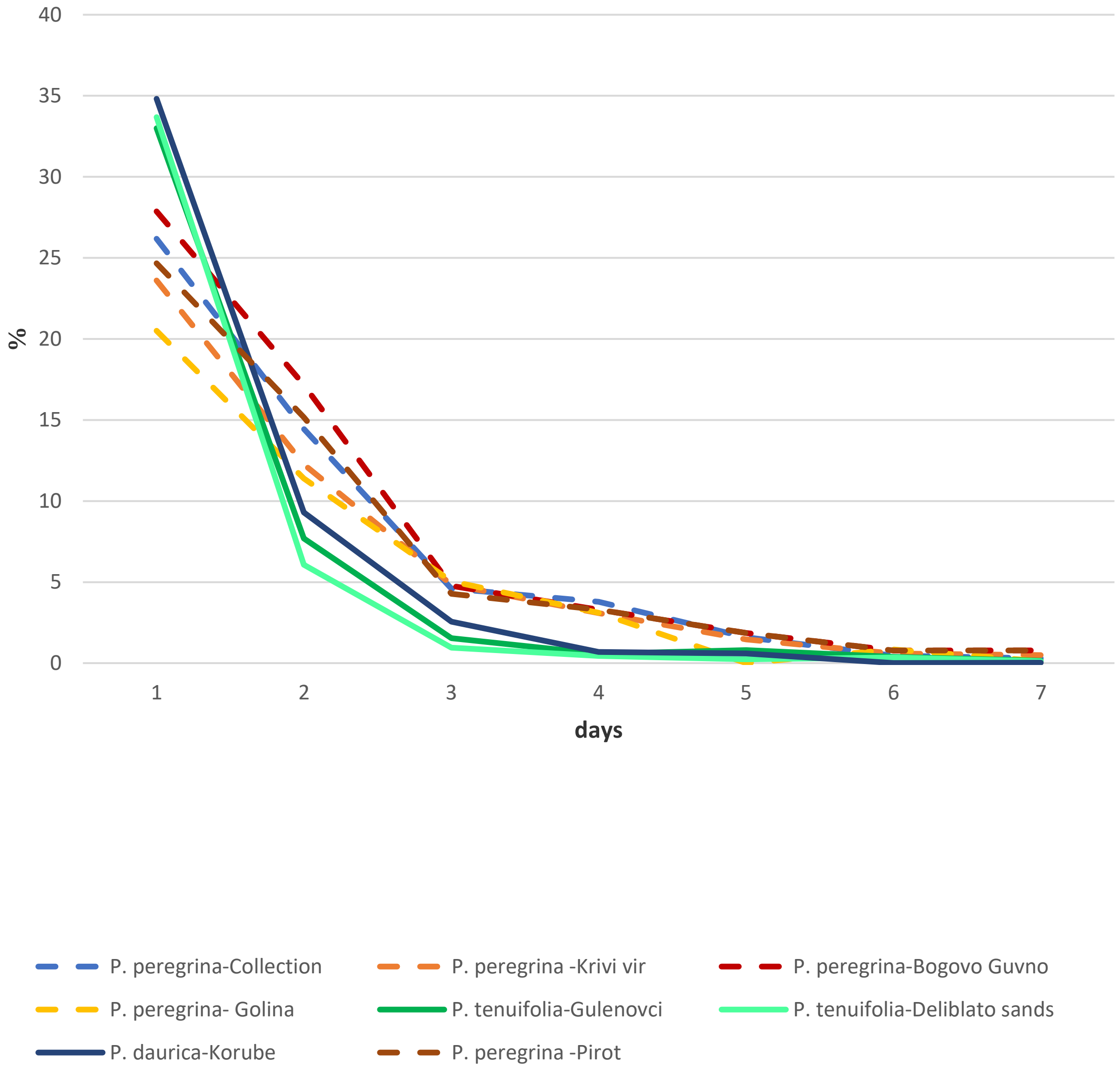
According to research on several plant species (Giles, 1990; Simpson et al., 2021) larger seeds within a species have higher germination energy and bigger seedlings. According to our research on the seeds of herbaceous peonies, there was variety within the species in seed weight according to natural habitat, however the variation is not significant ($p > 0.05$). The variances can be attributed to environmental differences. A significant difference was observed, within a species, between seeds of *P. peregrina* from their natural habitat and seeds harvested in the Institute's collection, which is to be expected, due to better growing conditions and the lack of competition. A significant difference was observed between *P. peregrina* and *P. tenuifolia*, as well as between *P. tenuifolia* and *P. daurica*. Since the seeds of *P. tenuifolia* are the smallest, it was predictable (Hong, 2010). According to our results the species had greater influence on the seed weight than locality. Although the percentage of small seeds was not high for natural habitats, it was probably higher since 2021. year was particularly very dry. *P. tenuifolia* has a higher percentage of stunted seeds, although it tolerates such habitats better, reason could be that in the initial stages of seed development was a particularly dry. Research of Sehgal et al., (2018) indicate that seed productivity might be decreased if early stage of seed maturity is affected by drought.

Table 3. Herbaceous peony seed weight according to species and locality

Species	Locality	Seed weight (g)	Undeveloped seeds (%)
P. peregrina	Pančevo	0.190 ± 0.025	3
	Golina	0.148± 0.023	3
	Krivi vir	0.140 ± 0.024	4.5
	Pirot	0.135 ± 0.047	4
	Bogovo Guvno	0.127 ± 0.025	3
P.tenuifolia	Deliblato Sand	0.051 ± 0.012	5
	Gulenovci	0.061 ± 0.009	5.5
P. daurica	Korube	0.150 ±0.031	3.5

Imbibition capacity

Peony seeds have a hard coat that must be softened and moistened for germination (Yu et al., 2007). The seed coat becomes hydrated during imbibition, allowing water to enter the seed, activating numerous enzymes and initiating metabolic processes that lead to seed germination. Imbibition is the initial stage of seed germination and is essential for plant growth and development. Water soaking has been reported to improve seed germination in both tree and herbaceous peonies (Yu et al., 2007).



Graph 1. Percentage of absorbed water of tested herbaceous peony species from different localities of its initial weight measured every 24 hours for seven days

An herbaceous peony's imbibition period usually lasts differently for different species. According to our findings, the seeds of *P. daurica* and *P. tenuifolia* absorb water more quickly than *P. peregrina*. *P. daurica* seeds absorbed water approximately 35% of their initial weight in the first 24 hours, while *P. tenuifolia* seeds absorbed more than 30%. *P. peregrina* absorbs water more slowly, averaging about 24% in the first 24 hours. *P. peregrina* imbibition capability differs by locality after the first 24 hours, which could be attributed to the initial seed moisture. Dry seeds absorbed the water slower. In the first 24 hours, seeds from the area with lower seed moisture at the beginning of the experiment absorbed water less on average, but there was equalization in the following period. By measuring individual seeds, it was determined that heavier seeds absorb more water, but if it is calculated as a percentage of initial weight, the results showed that the percentage of water uptake was related to the initial weight of the seeds. On the second day, *P. peregrina*, *P. tenuifolia*, and *P. daurica* absorbed 14%, 7%, and 8% of initial weight in water, respectively. On the third day, all species absorbed less than 5% of water. *P. peregrine* on fourth day absorbed 4% water on average, while the other two species absorbed less than 1%. In the following days, all species absorbed less than 1% of water. From the foregoing, it may be proposed two to three days is the optimal time for imbibition of *P. peregrina* and one to two days for *P. tenuifolia* and *P. daurica*.

Conclusion

Through the study of herbaceous peony species, an effort was made to ascertain the potential causes of the species' endangerment in their native habitats. As far as seeds are concerned, in herbaceous peonies, over 94% of the seeds are well developed within the species, and no influence of habitat was found; thus, one of the causes for the vulnerability is not the lack of production of quality seed. According to our findings, species had a greater influence on seed weight than locality. According to our research, two to three days is the optimal time for imbibition of *P. peregrina* and one to two days for *P. tenuifolia* and *P. daurica.*

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Quality of cucumber seedlings grown in different substrate volumes

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Abstract

The production of vegetable seedlings is a part of horticultural production that is very intensive and requires a number of horticultural practices and measures. The standards of quality seedlings are not well defined, but mostly refer to the following facts: without infections of diseases or pests, ability to survive in unfavorable environments after transplanting, well developed root system, adequate root:shoot ratio, and well developed leaf area without visible physiological defects of leaves. In this study cucumber seedlings (*Cucumis sativus* L.) were grown in five different substrate volumes (100, 200, 300, 500 and 1000 cm^3) with aim to determine which substrate volume ensured production of seedlings with the highest quality in the shortest time. Research was conducted in controlled conditions (growing chambers) at the Faculty of Agriculture in Belgrade. The quality of the seedlings was monitored through the following growth parameters: plant height (cm), stem height (cm), stem diameter (cm), number of leaves, plant fresh weight (g), and leaf area per plant (cm^2). The results obtained show that the increase in substrate volume resulted in a linear increase in the values of all measured quality parameters of the seedlings. However, the seedlings grown in a smaller substrate volume (especially 200 and 300 cm^3) also had growth parameters that met the standards for high-quality seedlings, which is economically justified from several aspects: lower substrate consumption, greater number of pots per unit.

Key words: cucumber, seedlings, substrate volume, plant height

Introduction

The production of vegetable seedlings is a part of horticultural production that is very intensive and requires a number of horticultural practices and measures. Well-nourished seedlings are the main precondition for successful vegetable production, especially for a high yield and its good quality (Prunty et al., 2015). However, according to the Balliu et al. (2017), the standards of high-quality seedlings are not precisely defined, but mostly refer to the following facts: without infections of diseases or pests, ability to survive in unfavorable environments after transplanting, well developed root system, adequate root:shoot ratio, and well developed leaf area without visible physiological defects of leaves (chlorosis or necrosis).

In modern vegetable cultivation, seedlings are grown mainly in a protected area, in containers or pots (Nichols, 2013). In this case, the choice of substrate is of key importance, and in addition, special attention is paid to the water and fertilizer regime, as well as environmental conditions. The role of substrate are to provide the plant with physical support, a suitable conditions for root growth and development, aeration, water and an adequate amount of plant nutrients (Olle et al., 2012). Depending on local availability, various organic (peat, compost, coconut coir, rice husks, tree bark) and inorganic (perlite, clay, vermiculite and mineral wool) raw materials are used to produce the growing media-substrate (Schmilewski, 2009). In the production of vegetable seedling plants are often used industrial growing mixtures adapted to the nutritional regime for the corresponding phase of production.

The cucumber (*Cucumis sativus* L.) belong to the family Cucurbitaceae which include about 960 species. The center of origin of this species is the Asian continent, more precisely India, while China is mentioned as a secondary center of origin (Valcárcel et al., 2018). Cucumber is a highly valued vegetable plant grown worldwide for its immature fruits, which are used in human nutrition as fresh in various salads or processed into pickles.

During 2021, cucumber was grown on more than 2 million ha, and the total annual yield was over 93.5 million tons. Of the total area under cucumber cultivation, 89% is in the Asian continent, with China being the top producer, while areas in Europe account for 6.6% of total production. In Serbia, the area under cucumber cultivation in 2021 was 2.769 ha, and the total annual yield was 29.177 tons (FAOStat, 2021).

Because cucumber and other members of Cucurbitaceae family have a root systems that are sensitive to transplanting, i.e. do not tolerate or regenerate well from any damage, the production of seedling is specific (Melo et al., 2016). In this regard, this study investigated the effect of five different substrate volumes (100, 200, 300, 500, and 1000 cm^3) on the quality of cucumber seedlings monitored through quality parameters (plant height, stem height, stem diameter, number of leaves, plant fresh weight, and leaf area). The aim of the study was to determine which substrate volume ensures the production of seedlings with the highest quality in the shortest time and at the same time is economically justifiable.

Material and Methods

The experiment was carried out at growing chambers at the Faculty of Agriculture, in Belgrade. For research purposes, cucumber seedlings (*Cucumis sativus* L, hyb. *Darina* F1) were grown in polypropylene pots filled with commercial substrate TKS 1 (Floragard) in five different volumes: 100, 200, 300, 500, 1000 cm^3. The experiment was carried out in triplicate. During the experiment the following conditions and measures were provided:

- air temperature of day/night cycle was 25/18^{o}C;
- during the whole experiment the day/night interval was 14/10 h. Artificial light was provided by lamps (Philips MH 600W);
- from sowing until the appearance of the first permanent leaf, 50 ml of water was added to each pot, and then the seedlings were watered with 70 ml of water until the end of the experiment;

After completion of the experiment (22 days after sowing), in the cucumber seedlings the following growth parameters were measured:

1. Plant height (cm) was measured from root collar to the top of the highest leaf, using meter scale;
2. Stem height (cm) was measured from root collar to the top of the stem, using meter scale;
3. Stem diameter (cm) was measured under the cotyledon leaves, with a digital caliper;
4. Plant fresh weight (g) was determined by weighing above-ground part of plants (stem and leaves) by scale (AXIS, model No. AD200, Poland);
5. Number of leaves was defined by counting leaves;
6. Leaf area per plant (cm^2) was measured by computer program ImageJ (Abramoff et al., 2004).

Significant differences between cucumber seedlings grown in different substrate volumes were estimated using one-way analysis of variance (ANOVA) with least significant difference (LSD) tests, at the $p<0.05$ and $p<0.01$ significance levels, by using RStudio software (version 2022.07.1) (RStudio Team, 2020). The results were expressed as mean and arranged in tables.

Results and Discussion

Table 1 shows the average plant growth parameters (plant height, stem height and stem diameter) corresponding to five different substrate volumes.

Table 1. Growth parameters of cucumber seedlings (plant height, stem height and stem diameter) produced in different substrate volumes

Substrate volume (cm^3)		Plant height (cm)	Stem height (cm)	Stem diameter (cm)
100		13.26	3.94	4.91
200		13.52	4.66	5.53
300		14.26	4.62	5.86
500		17.72	6.63	6.57
1000		21.30	7.76	6.59
LSD	$p \leq 0.05$	**1.77**	**0.82**	**0.51**
	$p \leq 0.01$	**2.42**	**1.12**	**0.70**

The values of plant height, stem height, and stem diameter of cucumber seedlings increased linearly with increasing substrate volume. The heights of plants grown in pots with substrate volumes of 100, 200, and 300 cm^3 were not statistically significantly different from each other, but they were statistically significantly differ ($p \leq 0.01$) compared to plants grown in substrate volumes of 500 and 1000 cm^3. In addition, there was a statistically significant difference between the height of the plants produced in the 500 and 1000 cm^3 volume substrates. Also, in the case of stem height, there was no statistically significant difference with smaller substrate volumes (100, 200 and 300 cm^3), while the difference in stem height measured in plants grown in pots with substrate volumes of 500 and 1000 cm^3 was statistically significant ($p \leq 0.01$). As for stem diameter, the lowest value was observed in pots with a substrate volume of 100 cm^3, which was statistically significantly lower ($p \leq 0.05$) than the stem diameter measured in plants grown in other substrate volumes (200, 300, 500, and 1000 cm^3). The stem diameter achieved at substrate volumes 200 and 300 cm^3, as well as substrate volumes of 500 and 1000 cm^3 did not differ statistically significantly.

Number of leaves, plant weight, and leaf area of cucumber seedlings grown in five different substrate volumes (100, 200, 300, 500, 1000 cm^3) are shown in Table 2.

The number of leaves, plant fresh weight and leaf area increased in a linear way with the increase in substrate volume.

The lowest number of leaves (2.00) was obtained in seedlings grown in pots with substrate volume of 100 cm^3, and the highest number of leaves (4.00) in the pots with maximum tested substrate volume (1000 cm^3). However, there was no statistically significant difference between the analyzed groups of cucumber seedlings. The volume of the substrate had a statistically very significant effect on the plants fresh weight, except for cucumber seedlings grown in substrate volumes of 200 and 300 cm^3, where no statistically significant difference was found. In the case of leaf area, there was also no statistically significant difference between the groups of cucumber seedlings grown in substrate volumes of 200 and 300 cm^3, while between the other groups there was a statistically significant difference, especially between seedlings grown in the minimum (100 cm^3) and maximum (1000 cm^3) tested substrate volume.

Table 2. Number of leaves, plant weight (g), and leaf area (cm^2) of cucumber seedlings grown in different substrate volumes

Substrate volume (cm^3)		Number of leaves	Plant fresh weight (g)	Leaf area (cm^2)
100		2.00	5.86	136.0
200		3.00	7.11	148.1
300		3.00	7.34	169.3
500		4.00	12.52	282.2
1000		4.00	15.64	372.2
LSD	*p<0.05*	**2.49**	**1.37**	**31.37**
	p<0.01	**3.92**	**1.87**	**42.79**

In research of Mello et al. (2016) were cucumber seedlings grown in substrate volume of 30, 60, 120 and 180 cm^3 , the results indicate that with the increase in the volume of the substrate, the quality of the cucumber seedlings also increases. Namely, according to growth parameters (shoot length, root length, root volume) the cucumber seedlings produced in the volumes of 60, 120 and 180 cm^3 were rated as high quality, while the seedlings in the smallest substrate volume (30 cm^3) were of unsatisfactory quality. Similar results were obtained in the research by Bjelić et al. (2010), in which the effect of substrate volume on the quality of pepper seedlings was investigated. These results can be explained by the fact that in the larger volume of the substrate there is a greater amount of water and nutrients, and at the same time there is more space for the growth and development of the root system.

Furthermore, similar studies indicate the optimal volume of container cells or pots for production of vegetable seedlings: 72 cell container (about 50 cm^3) for cucumber (Costa et al., 2012), also for eggplant (Costa et al., 2013) and tomato (Rodrigues et al., 2010). Certainly, when choosing the volume of the substrate, should take into consideration the vegetable species to be grown, the duration of cultivation in containers or pots, as well as the economic justification.

Conclusion

Cucumber (*Cucumis sativus* L.) is a nutritionally and economically important horticultural crop. The first stage of cucumber production is the production of seedlings, and the success of production depends on their quality. Besides the basic conditions (temperature, humidity, light, water), the choice of substrate and the volume of the pot are the most important. In this study, it was found that cucumber seedlings grown in pots with a larger substrate volume had higher values of the growth parameters studied (plant height, stem height, stem diameter, number of leaves, plant weight and leaf area) than plants grown in smaller substrate volumes. More precisely, the values of the mentioned growth parameters of the cucumber seedlings increased linearly with increasing substrate volume. Although the cucumber seedlings showed the highest values of the growth parameters when grown in the largest substrate volume, seedlings grown in a smaller substrate volume (200 and 300 cm^3) also had growth parameters that met the standards for high-quality seedlings. These points out the numerous advantages of growing cucumber seedlings in pots of smaller volume: lower substrate consumption, greater number of pots per unit, especially in protected area, and particularly greater economic profitability.
The idea is certainly to continue to expand research to introduce more hybrids into production and use more different substrates.

Aknowlegment

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Influence of photosynthesis inhibitors on chlorophyll content in redroot pigweed and lettuce

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Abstract

The aim of this study was to determine the effect of different concentrations of herbicides inhibitors of photosynthesis, , on the chlorophyll content two test plants: redroot pigweed (*Amaranthus retroflexus* L.) and lettuce (*Lactuca sativa* L.). Two herbicides Dancor 70 WG (a. s. metribuzin 700 g/kg) and Goltix 70 SC (a. s. metamitron 700 g/L) were applied. Five different concentrations were used and compared with the untreated control. The relative content of chlorophyll in the leaves of the treated plants was measured by chlorophyllometer SPAD 502. In both test plants, significant phytotoxic reactions were observed 7 days after herbicides application. Phytotoxicity on redroot pigweed manifested in the form of burning of the edge of the leaf and twisting towards the reverse side, depigmentation and drying of smaller leaves. On the lettuce, necrosis appeared from the top to the base of the leaf. The regression relationship between the percentage content of chlorophyll in the leaves of redroot pigweed and the applied concentration of both herbicides corresponds to a linear regression function (decreasing function), and for lettuce this relationship corresponds to an exponential function of the first order (decreasing function). Applied effective doses of metribuzin and metamitron showed that lettuce is more sensible than redroot pigweed. Also, a.s. metribuzin is more effective than a.s. metamitron in the reduction of chlorophyll content, for both researched species.

Key words: metribuzin, metamiton, redroot pigweed (*Amaranthus retroflexus* L.), lettuce (*Lactuca sativa* L.), chlorophyll content

Introduction

Metribuzin (4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one) is herbicides used to control broadleaf and grass weeds pre and post emergence in cereals, potatoes, tomatoes, peas, asparagus and lentils. Acts selectively, systemically with contact and residual activity (Lewis et al., 2016). Metribuzin inhibits all Hill reactions on photosystem II when water is the electron donor. Inhibit the photosynthetic flow of electrons between the primary and secondary acceptors (Q and plastoquinone) (Trebst & Wietoska, 1975). The electron-blocking causes reduced carbon dioxide fixation and production of ATP and NADPH2, which are known as energy packets of respirations. The action of photosynthesis inhibitors herbicides is greater during the daytime when there is full sunlight as the herbicide gets activated in presence of light. There are several symptoms of herbicide injury such as chlorosis and necrosis of leaf margins which progresses towards the base of the leaves after a few hours of application (Bagale, 2022). Metribuzin reduces the main fluorescence parameters of *Amaranthus retroflexus*, both in form of solution and in its incorporated form-granules (Volova et al., 2020). Moreover, slow-release formulations of metribuzin incorporated in poly-3-hydroxybutyrate [P(3HB)] proved to be the most effective, causing 100% mortality (Volova et al., 2020). Chlorosis of weeds leaves is a common side effect after the application of metribuzin, while it causes minimal damage in the range of 0-2.5% on the potato crop (Kalkoran et al., 2021; Renner & Powell, 1998). Effective suppresion (> 90%) of *A. retroflexus* in the potato crop can be achived with doses lower than indicated on the label, thus reducing production costs. For *A. retroflexus* satisfactory control efficacy (>90%) was observed with 240.8 g a.i. ha^{-1} at the BBCH 12-15 growth stage and with 293.2 g a.i. ha^{-1} at the BBCH 16-19 growth stage (Kahramanoğlu & Uygur, 2010). Metribuzin can also be mixed with other herbicides in glyphosate-tolerant corn crop, to reduce the number of glyphosate treatments. For example, metribuzin in combination with other herbicides is used for successful control of *A. retroflexus*, *Ambrosia artemisiifolia* and *Chenopodium album* (Nurse et al., 2006). In the percentage expression of leaf necrosis, a synergistic effect of mesotrione added to metribuzin was observed, regardless of the fact that the individual effect of mesotrione alone is weaker than the individual effect of metribuzin (Abendroth et al., 2006). Testing metribuzin and its combinations with other photosynthesis inhibitors in corn tolerant to pre-em and post-em herbicides is great important, bearing in mind that it successfully controls the main weeds without damaging the crop (Richburg et al., 2019). Soybean injuries caused by the use of metribuzin in the early stages of plant development can be bridged and recovered later, producing even more pods and grains in them (Arsenijevic et al., 2021).

Metamitron (4-amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one) is used to suppress broadlife and grass weeds in sugar beet, beet and fodder beet crops. Acts selectively, absorbed by the roots and leaves and translocated by the xylem to all plant parts. Metamitron inhibit photosynthesis at photosystem II (Lewis et al., 2016). It can be used pre-em and post-em. It has a fast metabolism in sugar beet plants, which recovery quickly after injury through the detoxification of this herbicide (Abbaspoor et al., 2016). Metamitron in sugar beet in combination with mechanical weed management provides good control of weeds, especially *A. retroflexus* and *Ch. album* (Zargar et al., 2011). *Chenopodium album* as a common weed in the sugar beet crop may have a tolerance mechanism for metamitrone metabolism similar to sugar beet. The mechanism is rapid deamination to deamino-metamitrone, which has no herbicidal activity at normal concentrations. Deamination has been attributed to cytochrome P450 monooxygenase enzymes, which are known to mediate the detoxification of several herbicides (Aper et al, 2012). Therefore, the effectiveness of controlling other weeds using metamitron may depend on its metabolism in plants, as in the case of *Ch. album*.

The aim of this study was to examine the effect of herbicides inhibitors of photosynthesis and assess phytotoxicity level by determining ED_{25} and ED_{50}. Test plants were redroot pigweed (*Amaranthus retroflexus* L.), which is a widespread and highly competitive weed of agricultural crops, and lettuce (*Lactuca sativa* L.), on which these herbicides have a phytotoxic effect.

Material and Methods

The experiment was conducted in the greenhouse of the Faculty of Agriculture in Banja Luka, during September and Oktober 2021. The seeds of redroot pigweed (*Amaranthus retroflexus* L.) were collected during the summer in Banja Luka region. Lettuce seeds were comercial. There were prepared 45 smaller pots for each test plants, which were filled with a commercial substrate that had been previously moistened with water. A larger number of seeds were sown in each pot. After sowing, the test plants were watered every day, greenhouse was regulary ventilated. When the test plants were about 5 cm tall, thinning was done, and 6-7 plants of uniform growth remained in each pot. The day before the treatment, excess plants were removed, so that 5 of the most developed plants remained in each pot.

For the treatment were used two herbicides inhibitors of photosynthesis: Dancor 70 WG (a.i. metribuzin 700 g/kg) and Goltix 70 SC (a.i. metamitron 700 g/L). Application rate for Dancor was 0.5-1.5 kg/ha with water 200-400 L/ha. This corresponds to a preparation concentration of 0.125% - 0.75 %, i.e. an active substances concentration of 0.875 g a. i./L to 5.25 g a. i./L (Table 1). Application rate for Goltix 70 SC was 4 L/ha with water 200-400 L/ha. This corresponds to a preparation concentration of 1-2 %, i.e. an active substances concentration of 7 g a.i./L to 14 g a.i./L (Table 2). Herbicides were applied by manual sprayer in five concentrations, and each concentration was done in four repetition. Each test plat had 4 pots that represented a control for both herbicides, which was compared with treated plants. The SPAD-502 meter is a hand-held device that is widely used for the rapid, accurate and non destructive measurement of leaf chlorophyll content. Measurements with the SPAD-502 meter produce relative SPAD meter values that are proportional to the amount of chlorophyll present in the leaf. In order to convert these values into absolute units of chlorophyll content, calibration curves must be derived and utilized. Converted SPAD values differ from photometric measurements of solvent-extracted chlorophyll by just 6% on average (Ling & Jarvis, 2011). Also, results of measurements with the SPAD device do not deviate from the results of measurements with other non-destructive devices, such as for example LEAF meters (Mendoza-Tafolla et al., 2019). Chlorophyll content was measured with a hand-held chlorophyll meter SPAD 502, 1, 2, 3, 4 and 7 days after herbicide application In the case of redroot pigweed, for each concentration, the measurement was performed on three pots and on all five plants in the pot. In lettuce case, measurement was done on five leaves in each pots.

Table 1. Concentrations of herbicide Dancor 70 WG (a.i. metribuzin 700 g/kg)

Mark	Concentration of herbicide Dancor 70 WG		Concentration of active substance (g/L)
	(g/0.2 L)	%	
K1	0.125	0.0625	0.437
K2	0.25	0.125	0.875
K3	0.5	0.25	1.750
K4	1	0.5	3.500
K5	2	1	7.000

Table 2. Concentrations of herbicide Goltix 70 SC (a.i. metamitron 700 g/L)

Mark	Concentration of preparation Goltix 70 SC (v/v)		Concentration of active substance (g/L)
	(ml/0.2 L)	%	
K1	0.5	0.25	1.75
K2	1	0.5	3.5
K3	2	1	7.00
K4	4	2	14.00
K5	8	4	28.00

Results and Discussion

The phytotoxic reaction on the treated redroot pigweed plants manifested in the form of leaf burning from the edges, which spread towards the middle of the leaf. Young leaves completly dried up. Leaf burn is not in the form of chlorosis, but in the form of a whitish or grayish color. Twisting of leaves toward the adaxial side was also observed (Fig. 1.).

Figure 1. A comparison of the appearance of control plants of redroot pigweed (right) compared to plants treated with the highest dose of metribuzin (left).

The relative concentration of chlorophyll was observed on the upper and lower leaves of redroot pigweed, which is shown in Table 3. In all measurement and with both herbicides, including the control plants, the relative chlorophyll content on the lower leaves was statistically significantly lower than on the upper leaves. Therefore, the regression relationship between the concentration of the tested herbicides and the chlorophyll content was considered only for the values measured on the upper leaf of redroot pigweed; and 7 days after treatments corresponds to a decreasing linear regression function.

Table 3. Chlorophyll content in the redroot pigweed leaf

Treatment	Concentration (g/L)	Leaf	1 day	2 day	3 day	4 day	7 day
Control	0	LL	29.9±2.3	28.6±0.6	28.0±2.2	30.7±1.3	24.5±2.8
		UL	33.9±0.7	34.5±1.3	31.9±0.8	31.4±1.0	36.0±0.8
Metribuzin	0.437	LL	29.4±0.4	26.1±1.9	27.3±2.1	27.6±1.6	23.9±1.9
		UL	31.2±2.5	32.7±0.4	31.6±2.1	30.7±1.9	27.9±2.9
	0.875	LL	26.7±2.4	28.8±0.4	26.5±0.5	28.6±1.1	27.7±1.0
		UL	32.6±1.2	31.2±1.5	33.4±0.7	30.9±1.1	26.7±2.4
	1.750	LL	26.3±2.7	25.3±1.9	25.2±1.0	27.7±1.9	19.1±0.9
		UL	30.8±0.9	31.4±0.2	30.2±1.3	31.7±0.6	26.5±1.7
	3.500	LL	25.6±2.2	25.5±1.5	28.6±1.0	29.7±1.3	24.7±2.7
		UL	30.6±1.6	28.2±2.7	28.0±2.8	27.8±3.7	24.7±2.1
	7.000	LL	27.4±0.9	27.1±2.4	26.0±1.8	20.5±1.9	23.1±2.1
		UL	28.6±2.1	30.9±0.5	30.9±1.1	31.0±1.3	19.1±2.3
Metamitron	1.75	LL	20.9±1.4	25.5±1.2	23.7±1.2	26.4±0.9	24.4±2.4
		UL	33.9±1.6	32.5±1.1	32.8±1.8	31.3±2.1	32.4±1.9
	3.50	LL	22.7±2.6	24.1±1.1	26.0±1.2	25.8±0.3	23.7±3.1
		UL	31.3±3.2	32.8±1.4	31.8±2.3	29.6±2.1	31.9±2.2

Treatment	Concentration (g/L)	Leaf	1 day	2 day	3 day	4 day	7 day
	7.00	LL	30.0±1.3	28.1±2.8	27.6±1.8	27.1±1.7	20.6±5.3
		UL	26.6±4.6	32.3±2.2	33.8±3.0	31.8±2.2	30.7±1.1
	14.00	LL	25.3±1.0	28.4±2.0	27.6±1.7	28.3±0.6	24.5±2.9
		UL	33.8±0.7	32.6±1.1	30.5±1.7	31.6±0.8	29.3±4.4
	28.00	LL	24.2±1.0	28.2±1.4	28.4±1.6	26.3±1.0	28.1±1.4
		UL	24.0±1.2	30.7±0.6	29.7±1.1	28.5±1.2	22.8±4.8
Legend: $\bar{x}$ ± SE=mean value ± standard error; LL= lower leaf; UL= upper leaf							

Phytotoxicity on lettuce was manifested in the form of necrosis, from the top to the base of the leaf (Fig. 2).

Figure 2. A comparison of the appearance of control plants of lettuce (left) compared to plants treated with the highest dose of metribuzin (right).

The regression analysis between herbicide concentration and chlorophyll content in the lettuce leaf 7 days after treatments corresponds to a decreasing exponential regression function of the first order.

Table 4. Chlorophyll content in the lettuce leaf

Treatment	Concentration	1 day	2 day	3 day	4 day	7 day
Control	0	18.0±2.2	15.0±3.0	15.4±2.3	18.2±4.1	16.9±2.7
Metribuzin	0.437 g/L	14.8±2.9	15.4±2.7	15.4±3.1	16.9±3.9	12.8±1.7
	0.875 g/L	14.2±1.6	15.4±1.4	14.3±1.9	14.3±1.8	12.3±1.9
	1.750 g/L	14.7±2.2	16.8±2.2	16.8±1.8	15.9±2.0	11.1±2.7
	3.500 g/L	13.8±1.7	13.9±0.3	13.5±0.3	13.5±1.6	10.0±1.9
	7.000 g/L	14.0±0.7	15.6±0.8	15.5±1.9	15.0±2.5	9.0±1.5
Metamitron	1.75 g/L	16.4±1.4	13.7±0.6	14.1±0.4	14.4±0.2	12.7±1.3
	3.50 g/L	17.3±1.4	14.3±1.5	14.9±1.5	18.1±2.3	12.5±2.1
	7.00 g/L	14.7±0.9	14.6±2.6	14.9±1.3	15.6±2.3	12.0±1.4
	14.00 g/L	11.8±0.4	12.2±0.4	11.1±0.7	13.3±1.2	11.4±1.0
	28.00 g/L	15.6±1.7	14.7±1.4	13.7±0.6	14.1±1.8	10.5±0.7
Legend: $\bar{x}$ ± SE=mean value ± standard error						

The comparative analysis of the effective doses herbicides was done using previously determined regression dependencies. As indicators of effective doses were taken ED_{25} and ED_{50} , i. e. herbicide concentration that reduce the chlorophyll content by 25, i. e. 50% compared to the control (Tab. 5.).

Table 5. Comparative analysis herbicides effective doses

Test plants	Metribuzin		Metamitron	
	ED_{25} (g/L)	ED $_{50}$ (g/L)	ED_{25} (g/L)	ED $_{50}$ (g/L)
Redroot pigweed	2,16	>7	16,19	>28
Lettuce	0,58	>7	1,84	>28

In our experiment metribuzin was highly effective in inhibiting photosynthesis when used alone, which is in accordance with Abendroth et al. (2006). It should be used in combination with other herbicides that inhibit photosynthesis in order to increase their synergistic effect and reduce selection pressure to post-em herbicides, as well as to determine the tresholds of metribuzin that provides a synergistic effect (Abendroth et al., 2006).

For better control, early application timing (BBCH 12-15 growth stage), when weeds are more sensitive, is necessary. To reduce costs of production, metribuzin should be applied in early stage of growth, when weeds are smaller and easier to control with lower doses (Kahramanoğlu & Uygur, 2010).

In addition to well-known mutation Ser-264-Gly (Aper et al, 2012), which leads to resistant biotypes of *Ch. album* to metamitron as before to atrazine, other mutations are also known. Mutation caused by supstitution at position 251 (Ala (from GCT)-251-Val(from GTT)) led to the creation of resistance *Ch. album* to metamitron, without previously established resistance to atrazine; and it is the fifth reported mutation that causes resistance to PS II inhibitors (Mechant et al, 2008). This indicate that genes causing resistance to PS II inhibitors are present in the environment, and that they can occur both on *Ch. album* and other widespread weed species such as *A. retroflexus* in our case. Research of resistance based on metabolism in weeds is significant for better weed control using metamitron in sugar beet and metribuzin in potato crop, especially if it is obvious that the weed is vital even after the treatment, and resistance has not been previously established (Mechant et al, 2008).

Conclusion

Applied effective doses of metribuzin and metamitron showed that lettuce is more sensibile than redroot pigweed. Also, metribuzin is more effective than metamitron in reduction of chlorophyll content, for both researched species.Although metribuzin showed high efficiency when applied alone, it can be recomended to mix metribuzin with other herbicides in order to delay the appearance of resistance, which is a pronounced and frequent occurrence with species belonging to the *Amaranthus* genus.

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About the Good Governance of Bulgarian agriculture

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Abstract

A new evolving concept of "Good Governance" has been increasingly debated in academic, governmental, business etc., circles. This paper tries to fill the existing gap suggesting a holistic framework for assessing the quality (goodness) of agrarian governance and estimating its level in Bulgaria. The assessment framework includes: defining the content and components of the agrarian governance system (Institutional Environment, Mechanisms and Forms of Governance, Process of Governing, Agents, and Systemic); formulating the principles of good agrarian governance (Good Legislation, Respectful Informal Rules, Good Working Public Sector, Good Working Private Sector, Good Working Markets, High Transparency, Good Involvement, High Efficiency, Good Leadership, Equity and Solidarity, and High Synergy); specifying the assessment aspects for each principle; identifying the best indicators for each aspect; selecting the criteria and reference values for assessing the quality of agrarian governance for each indicators; and deriving the good governance assessment score. The initial assessment using statistical and expert data found out that the Integral Governance Index of Bulgarian agriculture is at moderate level having in mind the EU perspective. The highest performance is attained under the principles of Equity and Solidarity and the Good Working Public Sector while in terms of the Working Private Sector and the Stakeholders Involvements it is the lowest.

Key words: good governance, assessment, agriculture, Bulgaria

Introduction

A "new" and constantly evolving concept of "Good Governance" has been increasingly used in the last three decades by the international, public, non-governmental and business organizations (AAID, 2008; ACML. 2020; DFID, 2010; Council of Europe, 2022; IFAD, 1999; OECD, 2015; World Bank, 2022), and is been a topic of "hot" academic debates of scholars in politics, economics, organization, development studies, international politics, behavioral sciences, socio-legal studies, etc. (Aguilera and Cuervo-Cazurra, 2019; Ali, 2015; Andrews, 2008; Bayyurt, Serin, Arıkan, 2015; Cheshire, Higgins, and Lawrence, 2007; Dasgupta and Roy, 2016; Fukuyama, 2016; Higgins and Lawrence, 2005; Narzary, 2015; Riegner, 2012; Steffek and Wegmann, 2021; Tripathi, 2017; Weiss, 2000). The critical role of the (good) governance in facing important (economic, social, environmental, etc.) challenges and achieving organizational, business, community, and social (including global) goals has been well recognized by the scientists, decision-makers, and public at large (Coase, 1991; Bayyurt, Serin, Arıkan, 2015; Ostrom, 2014; North, 1990; Williamson, 2005). Subsequently, attempts have been multiplying to specify and measure "how good or bad" that important factor of social development is. Furthermore, there is increasing acceptance that the good governance is a broader category than administration, business, economic, etc. efficiency, and (besides the Government) it is to include multiple agents and ("universal") social, environmental, etc. dimensions and goals. Thus, good governance is to be studied and assessed simultaneously as a means, a goal, and a result of "sustainable" socio-economic development (Bachev, Ivanov, and Sarov, 2020).

The major principles of "good" governance were initially introduced by the World Bank and become a benchmark related to "the manner in which power is exercised in the management of a country's economic and social resources for development". Since 1996 the Worldwide Governance Indicators have been reported annually including six governance dimensions: Voice and Accountability, Political Stability and Absence of Violence/Terrorism, Government Effectiveness, Regulatory Quality, Rule of Law, and Control of Corruption (World Bank, 2022). In addition, principles of "good" Corporate governance were introduced by OECD in 1999 including Discipline, Transparency, Independence, Accountability, Responsibility, Fairness, and Social Responsibility (OECD, 2015). Since its introduction, the content and principles of good governance have been specified, enriched, and widely adopted by international, governmental, business, non-governmental, and other organizations. In the EU a larger set of principles for good "regional" governance have been formulated, monitored, and enforced including Fair Conduct of Elections, Representation and Participation, Responsiveness, Efficiency and Effectiveness, Openness and Transparency, Rule of Law, Ethical conduct, Competence and Capacity, Innovation and Openness to Change, Sustainability and Long-term Orientation, Sound Financial Management, Human rights, Cultural Diversity and Social Cohesion, Accountability (Council of Europe, 2022). Subsequently, many of these principles have been enshrined in national laws and regulations and/or accepted as voluntary (organizational, business etc.) standards for behavior in the Union and beyond.

Despite its widespread use still, there is no consensus about the content of the good governance and a unified approach to its "measurement". There have been suggested and applied multiple methods for assessing the compliance with the principles (standards, codes, characteristics, dimensions, best practices, etc.) of good governance at global, regional, national, corporate, NGO, sectoral scales, at different functional areas of activity (e.g. internet, R&D, environmental management, etc.), and management of major resources (land, water, etc.) and social challenges (e.g. climate change, biodiversity preservation, etc.). Applied approaches for understanding and evaluating the system of governance mostly depend on the objectives of involved organizations and/or incorporated "methodological" frameworks. For instance, the assessments of the World Bank and some international and national donor agencies focus predominately on the public economic governance (extent of services provision, efficiency, corruption, etc.) in beneficiary countries; the framework applied by the EU, OECD, UN, and other organizations prioritize democracy, human rights, etc. aspects as well; the corporate sector puts primary attention on the safeguarding the of shareholders and (increasingly) stakeholders and social interests, etc. Similarly, political scientists and political economists are mostly interested in the "model" of governance and power relations, low scholars' study mainly formal legal "order", economists primarily investigate the (program, investment, transaction, third-party, etc.) costs and benefits, etc. The variation in the chosen "principles" and employed indicators for evaluating the "goodness" of governance creates confusion among different users and brings up criticism (Fukuyama, 2016). There is also a big criticism on applying a "Nirvana" approach which compares the real situation to some (Western, ideal, etc.) norms rather than to (an)other feasible "social arrangement(s)" (governance alternatives) in the specific conditions of a particular country, sector, region, agents, etc.

Another major reason for the lack of consistency in defining and assessing the quality of (good) governance is the diverse understanding of the concept of governance itself. Governance is defined in multiple ways but generally restricted either to governing bodies, agents, or groups (Hufty, 2011), or to the system of formal and informal rules and their enforcement (Ostrom, 2014), or to (certain) mechanisms, modes and structures of governance (Fukuyama, 2016; Weiss, 2000), or to the process of governing (Ali, 2015; Bevir, 2012; Hufty, 2011), or to the specific outcome and resulted social order (Bachev, 2010; Schmitter, 2018), or to the different combination of all them. Consequently, a big diversity of approaches and indicators are suggested and employed to evaluate the studied system of governance.

In the last years, there has been a growing number of publications evaluating the governance in the agrarian sector as well, including in certain countries, subsectors, food chains, types of farming organizations, functional areas, resources, territories, etc. All these studies demonstrate similar shortcomings as the general system of governance assessments and often are with poorly specified, uncomplete or contested definitions of governance, missing key components of the system of governance, lack of consistent framework for formulating principles and criteria for assessment, an arbitrary selection of indicators and references for measuring good governance, little adaptation to the specific conditions of a particular sector and level of analysis, etc. In Bulgaria, there are very few studies and assessments on the compliance to the principles of good governance of the public (Katsamunska 2016; EC, 2021; Stefanov, Yalamov, Mineva, 2016; Ganev, Popoca, and Bonken, 2020) and corporate (Dimitrov et.al., 2014; OECD, 2019) sectors. There are also several good studies on a particular type of agrarian governance - contractual, cooperative, institutional, environmental, food safety, etc. (Bachev, 2010, 2016; Boevsky and Sarov, 2017; Georgiev and Roycheva, 2017; Terziev et al., 2018). Nevertheless, with very few exceptions (Ivanov and Bachev, 2022, 2023), there have been no comprehensive study on the compliance of the agrarian governance in the country to the principles of "good governance" including all components of that complex system.

Adaptation of the interdisciplinary New Institutional Economics framework (North, 1990; Ostrom, 2014; Williamson, 2005) allows to overcome shortcomings of traditional approaches for understanding and assessing the system of governance as a whole and in agrarian sector in particular. It embraces all agents involved in the governing process and all mechanisms and modes that govern (structure, coordinate, direct, affect, manage) agents' behavior, actions, and relations (institutions, market, private, public, and hybrids). Furthermore, it applies the comparative institutional analysis in assessing and improving the existing system governance using not ideal reference norms (standards) but practically possible in the conditions of evaluated social system ("good governance") alternatives. This paper tries to fill the existing gap and respond to the great academic and practical (policies, business, and farming forwarded) issues suggesting a holistic framework for assessing the quality (goodness) of agrarian governance and estimating how good is the governance of Bulgarian agriculture at the present stage of development and EU CAP implementation.

Materials and Methods

The holistic framework for assessing agrarian governance includes several steps: defining the content and components of the agrarian governance system; formulating the principles of good agrarian governance; specifying the assessment aspects for each principle; identifying the best indicators for each aspect; selecting the criteria and reference values for assessing the quality of agrarian governance for each indicators; and deriving the good governance assessment score (Figure 1). Agrarian governance relates to the agricultural production and exchange, and involves all associated individuals and organizations - resource owners, entrepreneurs, farmers, downstream and upstream businesses, support agencies, communities, final, consumers, interests groups, policymakers, administrators, international bodies, etc. The system of agrarian governance consists of diverse mechanisms and modes that govern the behavior, activities, and relations of involved agents. The major components of the Governance system in agriculture include: (1) the institutional environment (formal and informal "rules of the game" and the system of enforcement of these rules); (2) Diverse mechanisms and forms of governance (market, contract, internal, collective, public, hybrid, etc.); (3) the process of governing (decision making, interacting, adapting to markets and demands, etc.); the agrarian and non-agrarian agents involved in the process – both governed and governing (Bachev, 2021). Agrarian governance is (to be) studied and evaluated at national, sectoral (crop, livestock), industry (grain production, horticulture), regional, ecosystem, type of farming organization (corporate, cooperative, family, semi-market, organic, etc.), major resources (lands, waters, etc.) and important challenges (climate change, biodiversity conservation, etc.) levels.

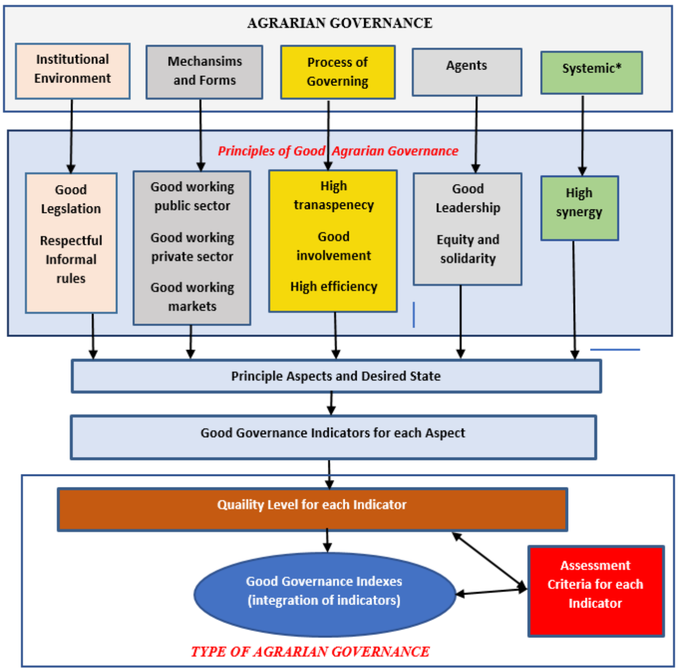

Figure 1. Framework for Assessing the Governance of Agriculture

*Systemic component is included since it determines the Governance as a system

Source: authors

Good Governance Principles are "universal" and relate to the best (desirable) state of the individual components of the governance system and the system as a whole. They are based on the widely accepted universal principle of good governance formulated by the international organization (EU, UN, FAO, etc.) and adapted to the specific conditions of agriculture. For instance, for the "specific" contemporary conditions of European Union (and Bulgarian) agriculture 11 (good governance) principles related to the individual component of agrarian governance have been selected by a Panel of Experts – Good Legislation, Respectful Informal Rules, Good Working Public Sector, Good Working Private Sector, Good Working Markets, High Transparency, Good Involvement, High Efficiency, Good Leadership, Equity and Solidarity, and High Synergy.

Aspects are precise standards ("measurement approaches") for each of the Principles representing a resulting state of the evaluated system when the relevant good governance Principle is realized. For contemporary Bulgarian conditions for every Principle 17 specific Aspects with their desired position have been identified by Panel of Experts – Supportive administration, No administrative deadweight, Efficient private sector, Accessible market, Fair competition, Confident level of awareness, Participatory decision-making, High return, Low transaction costs, High competency, Recognized promotion model, Gender equity, Fair distribution, High GAV agriculture, Stable employment, Competitive trade, and Resilient environment (Table 1).

Good Governance Indicators are quantitative and qualitative variables of different types which can be assessed in the specific conditions of the evaluated system allowing measurement of compliance with a particular Aspect. The set of Indicators provides a comprehensive picture of the state of individual components of agrarian governance and the system as a whole. For the selection of the Governance Indicators a number of criteria, broadly applied in the sustainability assessment literature and practices, were used: "Relevance", "Discriminatory power", "Analytical soundness", "Intelligibility and synonymity", "Measurability", "Governance and policy relevance", and "Practical applicability" (Bachev, Ivanov, Sarov, 2020). For the specific conditions of Bulgarian agriculture 36 indicators have been selected by the Panel of Experts (Table 1).

For assessing the particular goodness level, a system of specific Good Governance Criteria (best norms, range, standards, practices, etc.) for each Indicator are used. They are based on modern scientific research, European Union practices and standards, existing social contracts, etc. in the Bulgarian agriculture or in the evaluated subsystem of country's agriculture. Good Governance Criteria are the practically possible desired levels for each Indicator for the specific conditions of the evaluated agro-system. They assist the assessment of agrarian governance giving guidance for achieving (maintaining, improving) the best feasible standards for the particular components and the overall agrarian governance. Depending on the extent of the Criteria achievement the evaluated agro-system could be with a “good”, “satisfactory” or “bad” governance. For instance, a higher or similar to the EU level correspond to good governance for a particular indicator, and vice versa.

Assessment and analysis of compliance to the principles of good agrarian governance are done for each indicator. Very often individual Indicators for each Aspect and/or different Aspect and Principles of governance with unequal, and frequently with controversial levels. That requires a transformation into a “unitless” Governance Index and integration of estimates. Diverse quantitative and qualitative levels for each indicator are transformed into a Governance Index applying an appropriate scale for each Indicator.

Initial assessment of the governance of Bulgarian agriculture was done is the end of 2022 using data from statistical and other official sources as well as assessments of an 8-member Panel of Experts including leading scholars, and representatives of governmental and farmers organizations. The difference between used two types of indicators is the estimation modes, as the later ones is based on scores of Experts from a 5-level ranking scale (Very low, Low, Middle, High and Very high). The assessment score of each indicator is determined by the desired state derived from the principle aspects and indicator criteria interpretation, which means that in some cases, “Very low” is equivalent of 0, whereas in other cases might refer to 1. For the remaining indicators of governance, the Relative Comparison Assessment Method is employed (Ivanov, 2022). The statistically generated data are from different databases on macro and farm level, including Eurostat, FADN database averaging for 3-year period (2018-2020) whole experts’ judgments is done having in mind the recent years.

The common criteria used in this assessment is the average EU level and the medium EU situation. which is applied to provide the measurability and comparability of the assessment scores. The Good governance reference values are the practically observed indicators values on the counterpart EU average indicators. The later assist the assessment of agrarian governance giving guidance for achieving (maintaining, improving) the best feasible standards for the particular components and the overall agrarian governance.

Table 1. System of Principles, Aspcets, Indicators, and Criteria for Assessing the Quality of Governance of Bulgarian Agriculture

Principles	Aspects	Indicators	Estimation mode	Units
Good Legislation	Comprehensive legislation	Completeness of the legislation (1)	Experts assessment	Ranking score
	Justified enforcement	Degree of implementation and abide with legislation (2)	Experts assessment	Ranking score
		Level of regulation costs for get acquainted and to be enforced (3)	Experts assessment	Ranking score
Respectful Informal Rules	Mutual Trust	Level of trust between subjects in the agriculture (4)	Experts assessment	Ranking score
	Good Manner	Conflict level and contradiction state within agriculture community (5)	Experts assessment	Ranking score
Good Working Public Sector	No administrative deadweight	Level of unlawful payments and embezzlement (6)	Experts assessment	Ranking score
	Supportive administration	Satisfaction degree from administrative services (7)	Experts assessment	Ranking score
		Level of governmental spending for agricultural public administrating (agri-governmental expenditure unto total governmental spending) (8)	RCA method	Percent
Good Working Private Sector	Efficient Private Sector	Effectiveness of contracting among agents in agriculture (9)	Experts assessment	Ranking score
		Equality in the opportunities for development of different organizations forms (10)	Experts assessment	Ranking score
		Propensity to external contracting (contractual work to total output) (11)	RCA method	Ranking score
Good Working Market	Accessible market	Level of entry and exit market costs (12)	Experts assessment	Ranking score
	Fair competition	Competition fairness and avoiding price rigging (13)	Experts assessment	Ranking score
		Degree of market orientation (farm use and farmhouse consumption unto total output (14)	RCA method	Share
High Transparency	Confident level of awareness	Information awareness of stakeholders and agents in agriculture (15)	Experts assessment	Ranking score
		Costs level for information access of stakeholders and agents (16)	Experts assessment	Ranking score
		Decision-making transparency extent (17)	Experts assessment	Ranking score
		Symmetric between decisions taken and public expectations in agriculture (18)	Experts assessment	Ranking score
Good Involvement	Participatory decision-making	Plurality level in decision –making process in agriculture (19)	Experts assessment	Ranking score
		Level of unacceptable lobbying impairing third parties (20)	Experts assessment	Ranking score
		Scope of farm access to public agricultural support (% farms with direct payment/all farms) (21)	RCA method	Percent
High Efficiency	High return	Total spending of means and efforts for dealing with other economic agents and administration in agriculture (22)	Experts assessment	Ranking score
		Price rewarding potential (price index outputs/price input index) (23)	RCA method	Index
	Low transaction costs	Level of transaction costs in the agriculture (total farm overhead costs/total input) (24)	RCA method	Share
Good Leadership	Recognized promotion model	Level of achieving own advantage on the expense of others through legal and illegal means (25)	Experts assessment	Ranking score
	Recognized promotion model	Correctness and decency in the business relationships in agriculture (26)	Experts assessment	Ranking score
	High competency	Degree of competency and expertise of agents in agriculture (27)	Experts assessment	Ranking score
	High competency	Entrepreneurship abilities and level of self-improvement of agents (28)	Experts assessment	Ranking score

Principles	Aspects	Indicators	Estimation mode	Units
Equity and Solidarity	Ethnical, religious and bigotry equity	Level of discrimination on the ethnical, religious and bigotry causes (29)	Experts assessment	Ranking score
	Fair distribution	Fairness in the remuneration of employees in agriculture (compensation of employees/factor income) (30)	RCA method	Share
	Fair distribution	Balance in the public support distribution in agriculture (Gini coefficient) (31)	RCA method	Coefficient
High Synergy	Stable employment	People engagement in agriculture (share of population employed in agriculture) (32)	RCA method	Percent
	High GAV agriculture	Significance of agriculture in the economy (GAV of agriculture per capita) (33)	RCA method	Euro
	Competitive trade	Importance of agriculture in the trade (agriculture export/agricultural import) (34)	RCA method	Index
	Resilient environment	Contribution of agriculture to climate change mitigation (% of greenhouse gases from agriculture in total GHG) (35)	RCA method	Percent
	Resilient environment	Soil protection and control of nitrogen pollution (quantity of nitrogen fertilizers use) (36)	RCA method	Kg per ha

Source: authors

The Integral Governance Index is computed through weighting Principal score assessment based on the principle number and component count. The Integral Governance Index of Bulgarian agriculture is represented by a qualitative score, which ranges from 0 to 1 that might be converted into qualitative assessment. For the purpose of this research are formulated five categories that Governance Index implies: “very good’, “good”, ‘moderate”, “satisfactory” and “bad” governance. These qualifications are linked to: Index range 0,81-1 for a “Very Good” governance; Index range 0.56-0,80 for a “Good” governance; Index range 0,46-0,55 for a “Moderate” governance; 0,21-0,45 for a “Satisfactory” governance and Index range less than 0,20 – referring to ‘Bad or Unsatisfactory” agrarian governance. The governance assessment is oriented to the EU level, and therefore the Moderate rate is with a shorter range (plus or minus 0,05 deviation from the “average” EU value), while the extreme (Very Good or Bad) levels are kept in the normal 0.2 range in the 5 level Governance scale. Detailed explanation and justification of applied approach is done by Ivanov and Bachev (2023).

Results and Discussion

Initial approbation of the suggested framework has found out that the Integral Governance Index of Bulgarian agriculture is at moderate level having in mind the EU perspective. The highest performance is attained under the principles of Equity and Solidarity and the Good Working Public Sector while in terms of the Working Private Sector and the Stakeholders Involvements it is the lowest (Figure 2).

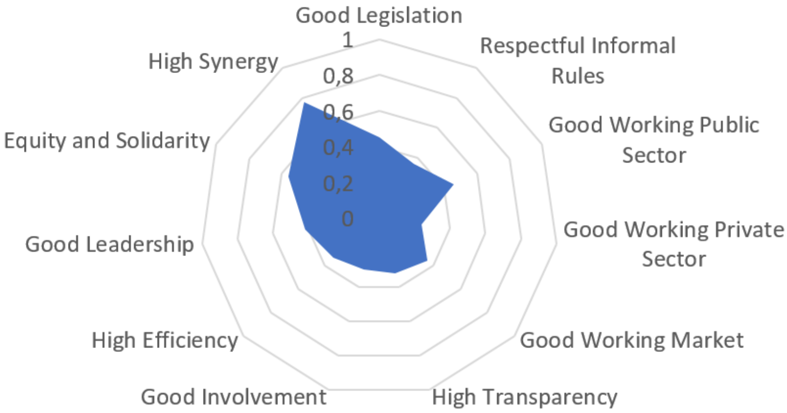

Figure 2. Level of Good Governance of Bulgarian Agriculture for Major Principles
Source: authors calculations

Analysis of individual indicators demonstrates that the strongest points of agrarian governance system in the country at the present stage of development are: Level of governmental spending for agricultural public administrating (agri-governmental expenditure unto total governmental spending), People engagement in agriculture (share of population employed in agriculture), Level of discrimination on the ethnical, religious and bigotry causes, Effectiveness of contracting among agents in agriculture, Importance of agriculture in the trade (agriculture export unto agricultural import), Degree of market orientation (farm use and farmhouse consumption unto total output), Completeness of the legislation, Level of regulation costs for get acquainted and to be enforced, and Correctness and decency in the business relationships in agriculture (Figure3).

At the same time, the weakest point of the governance system of Bulgarian agriculture are identified as: Propensity to external contracting (contractual work to total output), Equality in the opportunities for development of different organizations forms, Satisfaction degree from administrative services, Scope of farm access to public agricultural support (percent of farms with direct payment unto all farms), Level of trust between subjects in the agriculture, Symmetric between decisions taken and public expectations in agriculture, and Degree of competency and expertise of agents in agriculture. In all these directions the efforts of responsible officials, farm and agribusiness managers, professional organizations, and other stakeholders have to be directed though policies instruments, administration reforms, improvement of private and collective management, international assistance, etc. in order to improve the governance of agrarian sector in the country.

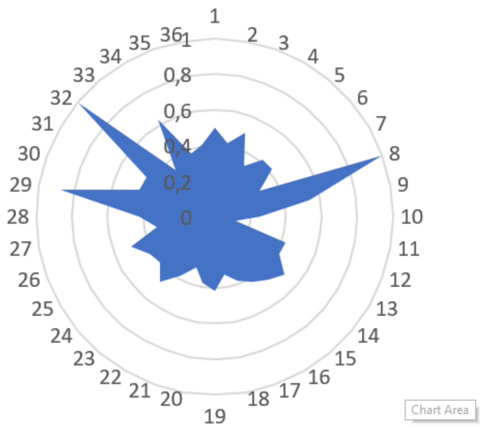

Figure 3. Level of Good Governance of Bulgarian Agriculture for Individual Indictors

Source: authors calculations

Conclusion

This study proved that the (quantitative) assessment of the governance system of Bulgarian agriculture and the level of its compliance to the principles of "Good governance" is possible. This has been just a first attempt to apply suggested holistic framework using available data and experts estimates. Therefore, further improvement of this "work in progress" is necessary both in terms of perfection of the hierarchical system of governance principles, aspects and indicators, its broader application into analysis of the governance system in major subsectors of Bulgarian agriculture (crop, livestock, etc.) as well as in appropriate data collection (including through official agri-statistics).

Besides, cooperation with the responsible government agencies, like Ministry of Agriculture and National Agricultural Advisory Service, professional organizations of farmers, and other stakeholders and interested parties is needed to perfect the evaluation system and apply is widely and periodically at various decision-making levels. Finally, international cooperation with researchers from other EU and neighboring countries is to be established to test suggested framework, make comparison between countries' agriculture and different subsectors, and further improve the system for assessing the good governance.

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Economic indicators for the sustainability of tobacco production in the Republic of North Macedonia

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Abstract

Tobacco production in the Republic of North Macedonia has a long tradition and is a strategic crop in the industrial crops sector, occupying a significant place in the structure of Macedonian agriculture and implying significant economic and social effects in the national economy. The overall goal of this research is to show the economic elements of sustainability of tobacco production as an indispensable industrial crop with equivalent economic benefits.

Through this paper, the need to study this issue will be shown due to the fact that tobacco production plays a key role both for the development of certain municipalities and for the entire national economy. The studies will mainly be based on secondary sources of data, and the set goal is dictated by several methods: method of indices, comparative inductive, deductive and other mathematical-statistical methods characteristic of agro-economic research. In Macedonia, tobacco production is organized on an average area of 16.260 ha, that is, 78.8% of industrial areas. About 22.000 producers are engaged in tobacco production, or rather, tobacco is a source of basic and additional income for a population of about 80.000 people. Tobacco production has a strong social component due to its labor-intensive nature, but it should also be emphasized that all stages of the production process are contractually regulated by a separate legal solution. Macedonia is among the top 30 tobacco producing countries in the world and among the 20 exporters of raw tobacco. Most of the tobacco produced in Macedonia, i.e. 90%, is exported. The fact is that Macedonia exported more than it imported in the analyzed seven-year period. Today, when the areas under tobacco maintain a stable level, the state support for the income of agricultural holdings engaged in tobacco production must continue, by encouraging balanced and sustainable development in the tobacco-producing regions, as well as support in the formation of producer organizations in accordance with European

experiences.

Key words: tobacco, unprocessed tobacco, tobacco production, sustainability, economic indicators, agricultural policy

Introduction

Tobacco production as an industrial crop mostly depends on climatic and soil factors. It is especially important to note that tobacco is also grown in conditions where no other crop can replace it. In conditions where the soils are of poor quality, tobacco can give a high yield and quality, while providing additional income. Since the Republic of North Macedonia has favorable natural agro-ecological conditions for tobacco production, mostly oriental type of tobacco is grown. It should also be emphasized that the Republic of North Macedonia has appropriate soil and climatic characteristics that are suitable for the production of individual varieties that have a unique character with a pronounced specific aroma and high quality of the raw material that is recognizable all over the world.

The average production of tobacco in the last seven years is 24.970 tons, that is, the average yield of tobacco for the analyzed period is 1.536 kg/ha. There are real possibilities for the total production of tobacco to stabilize, so that a large number of producers who grow tobacco for the realization of additional income would turn to tobacco production as a primary activity and a new source of livelihood.

In the past decade, the tobacco economy has undergone drastic changes compared to previous years. It is a known fact that Macedonia exports more tobacco than it imports, but the tobacco that is imported is tobacco that is not produced in this territory. In recent years, of the total quantities of purchased and processed tobacco, it belongs to privately owned companies (about 90%), while the rest of the tobacco is purchased by the Prilep Tobacco Plant, which is predominantly state-owned. The Macedonian tobacco sector is at a highly stable level, which is ensured through previously concluded contracts for the purchase of the produced tobacco, which is then forwarded and provides more effects and benefits to all stakeholders.

The Government of R. N. Macedonia adopted a seven-year strategy in accordance with EU directives. The Strategy contains the annual programs for financial support in agriculture and rural development that refer to state aid to agricultural holdings engaged in tobacco production, income support for agricultural holdings, balanced and sustainable development in tobacco producing regions and supporting the establishment of producer organizations.

Material and Methods

Taking into account the problems that this paper deals with, data obtained from regular statistical surveys conducted by the State Statistics Office, annual accounts from the Central Registry, and data from the Ministry of Agriculture, Forestry and Water Management were used. for the period from 2015 to 2021.

From the above data sources, relevant knowledge will be obtained about the areas under tobacco, the average yields, the total production of tobacco in the Republic of North Macedonia, as well as the value indicators for the exported and imported tobacco.

Results and Discussion

Areas under industrial plants for the period 2015-2021

Starting from the data representing the areas under tobacco and sunflower, and for the period from 2015-2021, shown in Table No. 1, we will note that a total of 144.478 ha were planted, or an average of 20.639 ha. It can be seen from the table that during the period tobacco was planted on an average of 16.260 ha, and sunflower on 4.380 ha. So, almost 79% of the total areas are planted with tobacco. The remaining 21% is accounted for by sunflower.

Table 1. Areas under industrial plants

Areas under industrial plants in ha.	2015	2016	2017	2018	2019	2020	2021	Total	Average	%
tobacco	16.128	16.379	15.961	16.582	16.719	16.592	15.457	113.818	16.260	78,78
sunflower	5.562	3.974	4.073	2.386	4.677	4.859	5.129	30.660	4.380	21,22
Total	21.690	20.353	20.034	18.968	21.396	21.451	20.586	144.478	20.640	100

Acreage and tobacco growers

Analyzing the planted areas with tobacco and the number of producers for the mentioned period (2015-2021), the first thing that is noticeable is that there are visible oscillations in the planted areas and that they have been decreasing in the last three years. At the same time, it is noticed that the number of producers is also decreasing. In the analyzed period, the average planted area is 16.260 ha, which is the closest to the area planted in 2016.

On average, 21.989 producers were engaged in tobacco production, regardless of the fact that the continuous decrease in the number of producers from year to year is visible.

If we look at the producers, the average planted area per producer is 0.75 ha, which is approximately to the average area per producer in 2018.

Table 2. Areas and producers of tobacco

Harvest	Planted area in ha	Number of manufacturers	Average area per producer, ha
2015	16.128	24.234	0,67
2016	16.379	23.438	0,70
2017	15.961	23.373	0,68
2018	16.582	23.426	0,71
2019	16.719	20.997	0,80
2020	16.592	19.702	0,84
2021	15.457	18.752	0,82
Average	16.260	21.989	0,75

Sown and harvested areas

From the table showing the planted and harvested areas with tobacco, it is evident that the largest area (16.719 ha) planted with tobacco is in 2019, when it is also harvested and the largest area (16.679 ha). The average planted area is 16.260ha, and the average harvested area is 16.253ha. So, with very small deviations, the planted areas were harvested.

Table 3. Sown and harvested areas in hectares

Year	Area in hectares	
	Sown	Harvested
2015	16.128	16.128
2016	16.379	16.376
2017	15.961	15.959
2018	16.582	16.582
2019	16.719	16.679
2020	16.592	16.591
2021	15.457	15.457
Total	16.260	16.253

Tobacco production and yield

In the production and yield of tobacco in the analyzed period, oscillations are visible both in the total production and in the obtained kg/ha. The highest production was recorded in 2019 of 26.234 tons, and the lowest two years earlier when it was 22.885 tons. In the same year (2017) is the lowest yield in kg/ha (1.434 kg.), and the highest in 2020 and 2021. The average production is 24.970 tons, and the average yield was 1.536 kg/ha. If in 2019, 26.234 tons were obtained from planted 16.719 ha, and in the previous year 25.547 tons were obtained from planted 16.582 ha, we will conclude that in 2019 the planted areas increased by 0.83% compared to the previous year, and the production increased by 0.97%.

Table 4. Production and yield of tobacco

Year	Tobacco production and yield	
	total, in tonnes	kg, per hectare
2015	24.237	1.503
2016	25.443	1.554
2017	22.885	1.434
2018	25.547	1.541
2019	26.234	1.573
2020	26.112	1.574
2021	24.329	1.574
Average	24.970	1.536

Purchase and average purcchase price of tobacco

As a result of the production, the purchase of tobacco was also carried out. From the attached data in Table No. 5, there are oscillations in the purchased quantities and therefore the average quantity of purchased tobacco is 24.167 tons. The average purchase price in the analyzed period moves with a slight increase, so that in 2020 it will come to a noticeable drop and in the following year to come again to a slight increase. In 2019, when the largest amount of tobacco was purchased (26.234 tons), the highest average purchase price per kg was reached (219,89 den. mkd). The average purchase price for the seven-year analyzed period is approximately 199 den. mkd per kg of tobacco, which is closest to the average purchase price in the last year. In table no. 5 we also have the values of the purchased tobacco, where it is noted that the average value of the purchased tobacco is 4.811.810,41 den.mkd or 89.248,51 US $.

Table 5. Purchase and average purchase price of tobacco

Year	Purchase and average purchase price of tobacco		Total value (000)	
	Realized purchase of tobacco in total, in tons	Average purchase price (den.mkd/kg)	den.mkd	US $
2015	18.910	184,5	3.488.895,00	61.816,00
2016	25.152	196,82	4950416,64	85.190,44
2017	22.885	217,6	4.979.776,00	97.527,93
2018	25.547	214,21	5.472.422,87	102.269,16
2019	26.234	219,89	5.768.594,26	105.323,98
2020	26.112	158,78	4.146.063,36	82.821,88
2021	24.329	200,44	4.876.504,76	89.790,18
Average	24.167	198,89	4.811.810,41	89.248,51

Export and import of row unprocessed tobacco of tariff number 2401

Most of the tobacco production, our country exports as raw, unprocessed tobacco. In the analyzed period, the smallest export of tobacco was in 2015 (22.775 tons) so that in 2021 the export amounted to 27.431 tons, which means that the export grew by 20.4% despite the drastic drop in 2020 when it amounted to 22.511 tons. In any case, the average export of tobacco is 25.179 tons each.

From the tabular display, the lowest export price was recorded in 2015 from 4.209 US$ per ton to reach 6.053 US$ per ton in 2020. So the export price grew by 43.8%, and the average export price was 5.234 US$ per ton.

For the needs of the tobacco industry, our country also imports certain types of tobacco. And in that case, oscillations of the imported quantities are visible. In the analyzed period, a total of 26.283 tons of tobacco were imported, or an average of 3.755 tons. Although fluctuations in import prices are visible, the required quantities of tobacco are imported at an average price of US$ 4.383 per ton.

Comparatively, the export is higher than the import as well as the average export price per ton is higher than the average import price.

Table 6. Export and import of raw unprocessed tobacco from tariff number 2401

Export				Import		
Year	Quantity in tons	Export price in US$ per ton	value in US $	Quantity in tons	Import price in US$ per ton	value in US $
2015	22.775	4.209	95.855.989	2.998	4.154	12.453.689
2016	27.692	4.265	118.119.734	5.945	3.863	22.963.886
2017	27.622	5.160	142.531.331	4.803	3.942	18.934.611
2018	23.327	5.908	137.816.042	4.703	4.594	21.606.459
2019	24.898	5.758	143.366.634	3.663	4.193	15.359.772
2020	22.511	6.053	136.252.966	1.872	4.804	8.993.395
2021	27.431	5.283	144.919.653	2.299	5.133	11.800.081
Total	176.256		918.862.349	2.6283		112.111.893
Average	25.179	5234	131.266.050	3.755	4.383	16.015.985

According to the National Strategy for Agriculture and Rural Development for the period 2014-2020 and the 5-year Program for the Development of Agriculture and Rural Development, measures and policies have been adopted to develop the tobacco sector, which should be in accordance with the policy of the Common Agricultural Policy in the EU as:

-Regulation (EU) No. 1307/2013 of the European Parliament and the Council of

December 17, 2013 for establishing rules for direct payments to farmers within the support schemes within the common agricultural policy,

-Regulation (EU) No. 1308/2013 of the European Parliament and the Council of December 17, 2013 on the establishment of a joint organization of markets in agricultural products,

- Regulation (EU) No. 1305/2013 of the European Parliament and the Council of December 17, 2013 on support for rural development by the European Agricultural Fund for Rural Development (EAFRD).

So, it can be concluded that the Republic of North Macedonia is aimed at the realization of:

1. measures and activities to increase the efficiency of the tobacco sector,
2. measures and activities for sustainable development of the tobacco sector,
3. measures and steps for adaptation of the tobacco sector of the Republic of Macedonia with the Good Agricultural Policy of the European Union (GAP)

In terms of increasing the efficiency of the tobacco sector as a leading direction towards the realization of the goals of the strategy, there is a need to support balanced and sustainable development and support for the formation of producers' organizations. The measures related to the Support for balanced and sustainable development of all tobacco regions are:

• Application of integrated measures for the production of tobacco and tobacco seedlings

  - procurement of protective equipment (to protect seedlings and tobacco from pests and weeds),
  - procurement of irrigation equipment and drip systems,
  - training of young tobacco producers,
  - legal and natural persons for the purchase of modern equipment, equipment for irrigation of tobacco in the field.

• Support for the production of tobacco seedlings by purchasing enterprises intended for individual tobacco producers,

• Support for conducting studies and research on the possibility of tobacco production as a renewable source of bioenergy for heat production,

• Support for the formation of producers' organizations for the joint production of seedlings

  - investments in the application of common production practices for compliance with standards,
  - purchase of common equipment for the production of seedlings,

• Support for the establishment of producers' organizations for the joint use of tobacco dryers,

• Support for the establishment of producer organizations for integrated tobacco production,

- formation of an organization of producers for joint procurement of pesticides, fertilizers, joint use of machines, soil analysis,
- Economic association of legal and natural persons for carrying out agricultural activity for integrated tobacco production and,
- introduction of common practices for the production of integrated tobacco production,

• Support of production of tobacco seedlings and cultivation of oriental tobacco in tall greenhouses - basic conditions for cultivation,

Regarding the measures and activities related to the sustainable development of the tobacco sector, the following will be implemented:

• measures and sub-measures that need to be implemented in order to achieve strategic goals for supporting the income of agricultural farms and state-aided farms engaged in tobacco production

- payments for performing agricultural activity in areas with limited opportunities for agricultural activity,
- support of collective investments,
- support for modernization and/or restructuring of agricultural holdings,
- support for the construction of wells and micro-dams (accumulations),
- support of operational groups

• measures and activities that should be implemented in order to achieve strategic goals for balanced sustainable development of tobacco production through environmental protection, introduction of "Good agricultural practice", efficient use of natural and other resources and economic development

- Specific minimum conditions for "Good agricultural practice" and soil protection,
- special minimum conditions for agricultural practice and water protection,
- Special minimum conditions for "Good agricultural practice" in plant protection,
- Agro-ecological aid for tobacco production,
- support in soil improvement,

- Support for protecting the soil from erosion,
- Support for the introduction of agricultural production with higher quality standards,
- Sustainable use of agricultural land,
- application of prescribed standards in the field of environmental protection,

• Measures and activities to increase competitiveness and maintain the traditional reputation of the tobacco market and they include:

- Financial support for demonstration-production trials among producers with newly created varieties of tobacco,
- participation in international gatherings of tobacco associations,
- publication of scientific articles on Macedonian tobacco in renowned secular magazines on tobacco, etc.

Regarding the measures and steps for adapting the tobacco sector of the Republic of North Macedonia to the Common Agricultural Policy of the European Union, they are aimed at realizing the strategic goal of supporting the income of tobacco farms through the following two approaches:

• Approach 1 – Additional national direct payments as a percentage of the financial package for direct payments, supplementing the percentage for the respective year with national principles from 30% to 100%. The state can determine, based on objective criteria and after authorization from the European Commission, the amounts of national participations, and

• Approach 2- The European Commission authorizes the new member state to supplement the percentage for the given year with additional national payments up to 100% of the size of the financial package.

From what has already been stated, it can be concluded that today agriculture, especially tobacco production, faces significant challenges in response to the rapidly changing global environment of agribusiness. Tobacco is the most profitable agricultural crop of all agricultural and industrial crops in the Republic of North Macedonia, taking into account the yield, purchase prices, subsidies and the regulated market. However, bearing in mind the intention of North Macedonia to be part of the EU, the preparation of tobacco farms, the compliance of all measures and activities in accordance with European directives is necessary.

Conclusion

Based on the analysis of the economic indicators of tobacco production in Macedonia, several conclusions can be drawn:

- tobacco production is organized on an average area of 16.260 ha, that is, 78.8% of industrially planted areas. It shows that tobacco is still considered an attractive culture. In that context, a specific mapping of tobacco producers who will work professionally and farmers who periodically approach tobacco production should be done. In that direction, efforts should be made to raise the awareness of tobacco producers regarding the opportunities offered by tobacco production,
- Although there is a noticeable decrease in planted areas, it is necessary to take appropriate measures related to maintaining and increasing the level of planted areas of tobacco,
- In the Republic of North Macedonia there are real possibilities for the stabilization of the total production of tobacco, which would increase the efficiency and quality of produced tobacco. In that direction, the liberalization of the negotiation system when concluding contracts for tobacco production is needed,
- If the purchase price of tobacco increases in real terms, then the motivation of producers to produce quality tobacco would also increase, and thus the value indicators would improve.
- The increase in the purchase price will create conditions for the production of higher quality tobacco, which is the main factor for a higher export price.
- The amount of imported tobacco that is necessary for the needs of tobacco manufacturing and tobacco processing maintains a level of reduction,
- The quantity of exported tobacco is only a confirmation of the demand in the international market
- North Macedonia, as a candidate country for EU membership, will have to adhere to the EU's Common Agricultural Practice (CAP) and full compliance with EU regulations.

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Z generation attitudes and opinions about beer

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Abstract

The aim of this research was to identify the key attitudes of Generation Z that influence the purchase, consumption and preference for domestic or foreign beers. The research was conducted in the second half of 2022. Primary data were collected online, using a structured survey questionnaire. The model is based on the opinions of 25 adult members of the Z generation who are beer consumers. The consistency of the received answers is within the range of acceptable limits. Quality and market characteristics of beer were selected as the two main criteria that influence consumer attitudes about beer. The term quality of beer includes the criteria of color, taste, smell, alcohol content and bitterness, and within the criteria of market characteristics sub-criteria are price, availability, awareness and packaging. Based on the Analytical Hierarchy Process method applied using the Expert Choice program, it was concluded that beer quality factors are more important than market characteristics factors. The most important factor when buying beer is taste, which influences the purchase with 28%, followed by bitterness at 16%, alcohol content at 13% and smell at 12% influence the purchase. Price affects 9%, color 8%, availability 6% and finally familiarity and packaging 4% each. Preferences of young beer consumers were calculated based on the weighted sub-criteria obtained on the basis of the attitudes of the respondents. Based on all the factors, young consumers are more committed to foreign beers compared to domestic beers in a ratio of 52:48%.

Key words: **b**eer, attitudes, opinions, Z generation, AHP

Introduction

The beer market and the motives behind beer purchases represent a broad field for primary research. Beer is an ideal beverage for different occasions - family gatherings and celebrations, holidays, going out (clubs, cafes), especially for outdoor events such as picnics, barbecues and the like. It is a typical working-class drink and is dominantly associated with society, socializing, a good atmosphere, fun, and relaxation (Ipsos, 2018). The beer market is not only an interesting sector to research but also provides important general economic insights (Swinnen, 2009). Despotović (2017) points out that beer consumption is increasing in low-income and middle-income countries with economic growth, such as China, Russia, Poland and India. In Bosnia and Herzegovina, the estimated consumption of beer is around 60 liters per capita (Ipsos, 2018). Bosnia and Herzegovina represents a specific market (due to the political environment) because it has three entities in which two beer brands appear as dominant brands (Jelen and "Ožujsko beer). The market is divided by regions, so each region has its own national brand, which is best positioned. In the Republic of Srpska, Nikšićko, Jelen and domestic Nektar are better-positioned brands, while in the Federation of Bosnia and Herzegovina, those are Sarajevo beer and Heineken, according to the same research. These brands are also competitors amongst each other in the market of Bosnia and Herzegovina (Vujičić, 2014). According to the report of the Chamber of Foreign Trade of Bosnia and Herzegovina (2019), it is stated that larger breweries, "Sarajevska pivara" p.l.c. Sarajevo, "Banjalučka pivara" JSC Banja Luka, "Bihaćka pivovara" p.l.c. Bihać and "Pivara Tuzla" p.l.c. Tuzla has been operating in Bosnia and Herzegovina for many years.

According to Hajdu et al. (2007), young people drink beer once a week, mostly from foreign brands such as Tuborg and Heineken. It is mainly based on quality and subject to the influence of foreign beer brands' promotion since they have the economic power needed for constant promotional activities. However, the research by Mandarić (2012) shows that consumer ethnocentrism contributes to the attitude of domestic consumers toward foreign brands. It has been found that consumer ethnocentrism tends to affect consumer perception of products from different countries. According to Chao et al. (2005), consumer patriotism and national animosity have been identified as influential emotional factors in the formation of consumer attitudes toward products from different countries as well as the intention to purchase.

The results of research by Batra et al. (2000) show that it is possible to expect a highly ethnocentric consumer to refuse to buy imported products because they consider it as unpatriotic decision, harmful to the domestic economy, etc. According to Wanninayake and Chovantsova (2012), consumer ethnocentrism has arisen from the sociological phenomenon called “ethnocentrism” which is “the technical name for a way of looking at things in which one's group is at the center of everything, and everyone else is scaled and judged against it”. The same authors, in their research, concluded that beer consumers in the Czech Republic have a strong love and pride for domestic, and an aversion to imported beers.

There are many factors that influence the world's consumption of beer - international and economic development and knowledge transfer, local traditions, climatic conditions, government regulations, religion, relative beer prices, other alcoholic beverages, etc. (Teichmannova, 2017). Quality in the customer's mind connects to the observation of the product, tangible and intangible. Perceived quality can be defined as the consumer's perception of the overall quality or superiority of the product or services in relation to the purpose or in relation to alternatives (Shariq, 2018). Brand perception is influenced by the level and intensity of presence in the media, propaganda messages and beyond the market environment (Šerić, 2009). The familiarity of the brand in the mind of the consumer is related to the strength of the brand and traces in the customer’s memory, which is reflected in the ability of consumers to identify the brand in different conditions and situations (Shariq, 2018).

In this paper, two main criteria were selected for the consumer's decision to purchase the beer, i.e. quality as a measure of the consumer's use value of the product and market characteristics (Jalić et al., 2022). Quality and its sub-criteria represent subjective, intrinsic factors, while market characteristics represent external, or extrinsic factors that are more measurable in relation to the aforementioned intrinsic factors. Based on the stated views and results of previous research, the current nature of the topic and the set research problem, the goal of this research follows, which was to investigate the most important factors that influence the purchase of beer by young consumers (Generation Z more specifically). But the goal has been also to determine their affinities when purchasing, that is, whether young consumers prefer domestic or foreign beers.

Materials and Methods

The research was conducted online using Google Forms which examined respondents' attitudes. Members of Generation Z (born from 1997 to 2012), young beer consumers, were targeted for the sample. Younger than 18 years could not be surveyed due to restrictions on alcohol consumption. The Expert Choice program in which the survey responses were processed allows up to 25 respondents, so the sample is based on 25 survey questionnaires in the Republic of Srpska. The first part of the questionnaire referred to basic sociodemographic questions, while the second part of the questionnaire includes all mutual comparisons of factors that influence the decision to buy beer. Survey questions were adapted to the survey method (Analytical Hierarchy Process - AHP method). Vindiš et al., 2010; Rozman et al., 2013; Pozderec et al., 2015; Pažek et al., 2014; Jalić et al., 2022; applied a multi-criteria assessment in agriculture production and food purchasing. The AHP includes questions based on Saaty's principles and a scale originally named after this researcher where the judgments are given in the form of paired comparisons (Saaty, 1990). An explanation of Saaty's scale is given in Table 1.

Table 1. Saaty's comparison table (Saaty, 1990; Jha, 2013)

Definition	***Explanation***	**Importance**
Equal importance	*Two activities contribute equally to the objective.*	1
Weak dominance	*Experience and judgment strongly favor one activity over another.*	3
Strong dominance	*Experience and judgment strongly favor one activity over another*	5
Demonstrated dominance	*Activity is strongly favored and its dominance is demonstrated in practice.*	7
Absolute dominance	*The evidence favoring one activity over another is of tile highest possible order of affirmation.*	9
Intermediate values	*When compromise is needed.*	(2, 4, 6, 8)

The mentioned scale has 17 potential answers, eight are on the left and eight are on the right side of the horizontal scale. Answer "1" implies indifference when comparing two factors that are located on the left and right. The linear scale in Google Forms enabled an efficient application of Saaty scales.

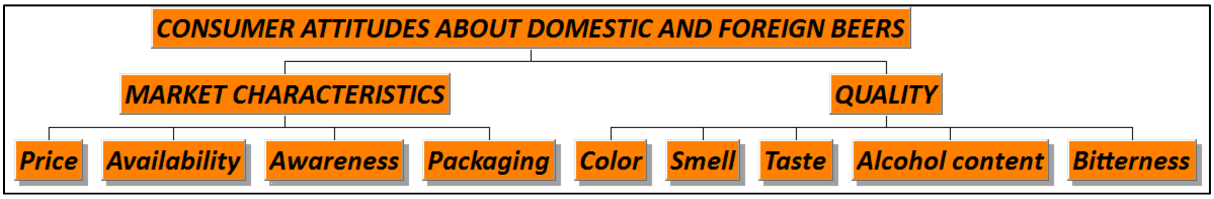

Figure 1. AHP hierarchy structure (*Authors' design generated in Expert Choice program*)

The questions were formulated based on the above showed factors (criteria) and sub-factors (sub-criteria). The scheme of the set model is shown in Figure 1, where it can be seen that Market characteristics and Quality represent the main criteria important for making a decision when buying beer. Price, Availability, Awareness and Packaging are sub-criteria for Market characteristics and Color, Smell, Taste, Alcohol Content and Bitterness are sub-criteria of Quality.

The conducted research was conceived in 6 steps and the framework of the research is shown in the picture below.

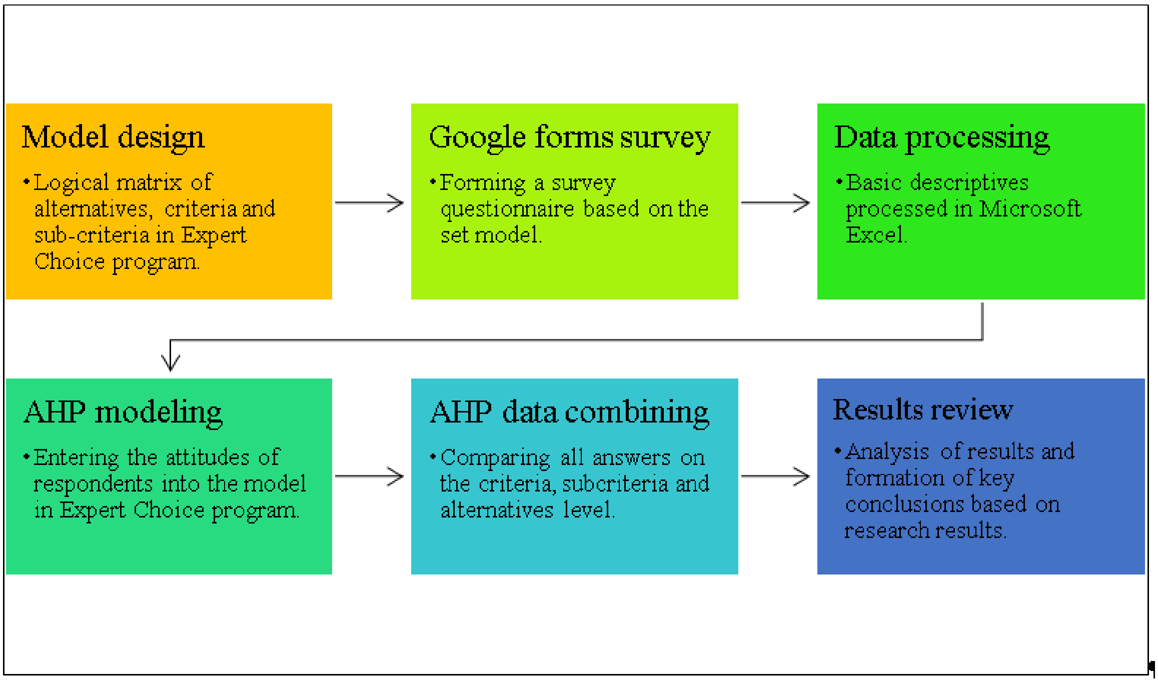

Figure 2. Research framework steps (*Authors' design*)

By studying the scientific and professional literature, information about the attitudes that influence the purchase and consumption of beer is gathered and the key criteria of the attitudes of beer consumers are identified. Based on quality factors and market characteristics of beer that were previously determined empirically by researchers who dealt with this topic, sub-criteria relevant to this research were determined, as well as alternatives determined by the goal and problem of this research. Then follows the formation of a survey questionnaire using the Saaty scale, as the most suitable for this type of research. The data collected by the survey questionnaire are initially processed using the basic methods of descriptive statistics, based on which conclusions are drawn about the basic characteristics of the sample. Also, the data from the survey questionnaire serve as inputs to the already constructed model in the Expert Choice program. Each individual answer contributes equally as an input to the overall constructed model of consumer attitudes. Based on the weighted influence of each of the sub-criteria, the final result of the conducted research is obtained - which beers are more attractive and desirable for the Z-generation adults based on the attitudes and determinations of the respondents.

Results and Discussion

After collecting the survey responses, the processing of sociodemographic data was initially started in Microsoft Excel, and the obtained results are shown in Table 2.

The variables gender, age, number of household members and monthly household income were selected as relevant in this paper. The gender structure is dominated by the male, which is inherent to beer consumption. The average age of the respondents is 22.2 years, the median is 22 years and the mode is 21 years with a very small range, of 8 years. Therefore, the age structure can be said to belong to the Z generation. In all of the indicators of central tendencies, the number of household members is about 4. Households usually have an income of over 3,000 BAM and from 1,200 to 1,800 BAM.

Table 2. Descriptives of the research sample

Variables	**Value**
Gender	
Female	*20%*
Male	*80%*
Age	
Mean	*22.2*
Median	*22*
Mode	*21*
Range	*8*
Household members	
Mean	*4.08*
Median	*4*
Mode	*4*
Range	*5*
Household monthly income (BAM)	
up to 1,200	*12%*
1,201 to 1,800	*28%*
1,801 to 2,400	*16%*
2,401 to 3,000	*12%*
more than 3,000	*32%*

Source: *Authors' calculation based on survey questionnaire*

All subsequent steps and the data processing were carried out in the Expert Choice program, i.e the Analytical Hierarchy Process was applied. The first stage of this method involves a comparison of the criteria and the other sub-criteria (Figure 3).

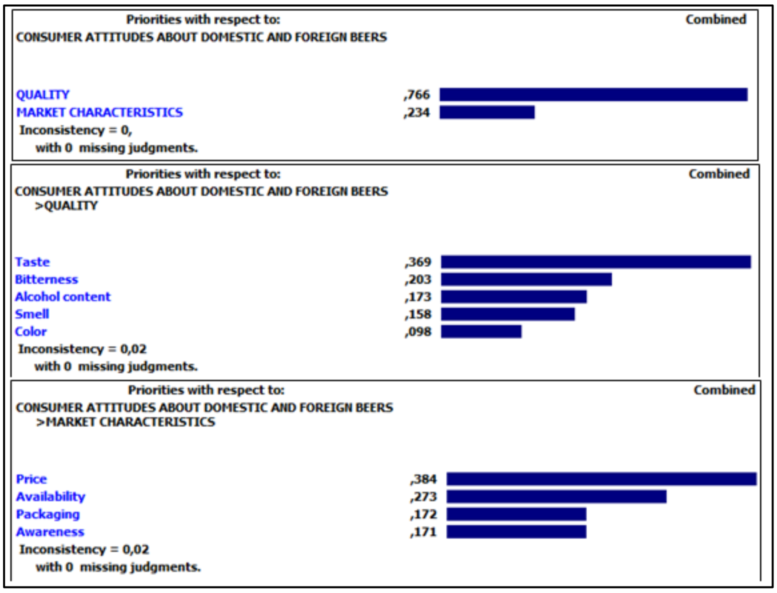

Figure 3. Determined priorities of Quality and Market characteristics and their sub-criteria (*Author's calculation in Expert Choice program*). **The punctuation mark "," in the Expert Choice program implies a decimal separator.*

The condition for the research to be valid is that the comparison of priorities of criteria and sub-criteria should be consistent. As a reliable level of permissible inconsistency, the value 0.10 is defined; in other words, if the inconsistency index is less than that value, the solution is considered acceptable and the evaluation process sufficiently consistent (Srđević et al., 2003). Based on the shown inconsistency indicator, it can be seen that this condition is met because the values of the main comparison (Market characteristics and Quality) are 0.00, and comparisons within the criteria of Quality and Market characteristics have an inconsistency factor of 0.02 each.

Based on the respondents' answers and the obtained results, it can be seen that the quality of the beer is more important to the Z generation in relation to market characteristics (76.6% to 23.4%) which coincides with the research of Hajdu et. al (2007) who stated that young people are mainly based on the quality of beer.

Within the **Quality criteria**, separately, the Taste sub-criteria stands out, with a value of 36.9%, followed by Bitterness at 20.3%, Alcohol content at 17.3% and Smell at 15.8%. The least important factor in the criteria beer Quality is sub-criteria Color with 9.8%.

Analyzing separately, the most dominant **Market characteristics** sub-criteria is Price, as expected, with 38.4%. The second most important factor is Availability, with 27.3%. Among the important factors in beer consumption, the price stands out due to its presence within the phenomenon of beer consumption among respondents (Thome et al., 2016). Packaging and Awareness equally affect the Market characteristics of beers (17.2% and 17.1%).

The following figure 4. shows the summarized weighted sub-criteria according to answers from 25 interviews. The blue sub-criteria belongs to Beer Quality and the yellow to Market characteristics of the beer. So the picture shows how much each sub-criteria affects the final consumer decision when buying and consuming beer. The corresponding value of the sub-criteria is obtained by multiplying the values of the main criteria and sub-criteria from Figure 3 (e.g. Price = 0.234*0.384= 9%).

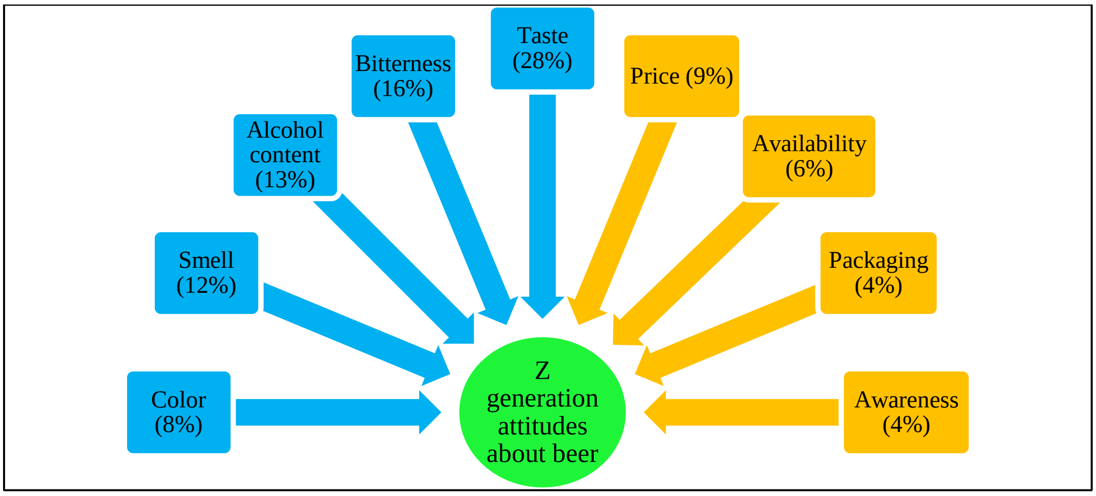

Figure 4. Subcriterias (factors) impact on Z generation attitudes toward beer purchasing and consumption

Surce: *Authors' design and calculations*

It has already been said that the Market characteristics of beer (Price, Availability, Awareness and Packaging) affect the purchase decision of beer by 23,4%, while Quality (Color, Smell, Taste, Alcohol content and Bitterness) affects the decision by 76,6%. By equating all sub-criteria to the same level, it can be concluded that the least influential factors in the purchase and consumption of beer are Awareness and Packaging, each 4%. Then Availability 6%, Color 8% and Price 9%. The next group of sub-criteria represents the factors of Smell, 12%, Alcohol content, 13% and Bitterness, 16%. The most important sub-criteria among others is Taste as a specific impression of drinking beer and has a share of 28%. That the most important factor when buying a beer is taste, Svatošová et al., 2021. confirmed with their research.

The last stage of the research was the determination of alternatives (domestic or imported beer) based on the selection of respondents on Saaty's scale and AHP criteria and subcriteria combining. Based on all the answers, the results are shown in the picture below.

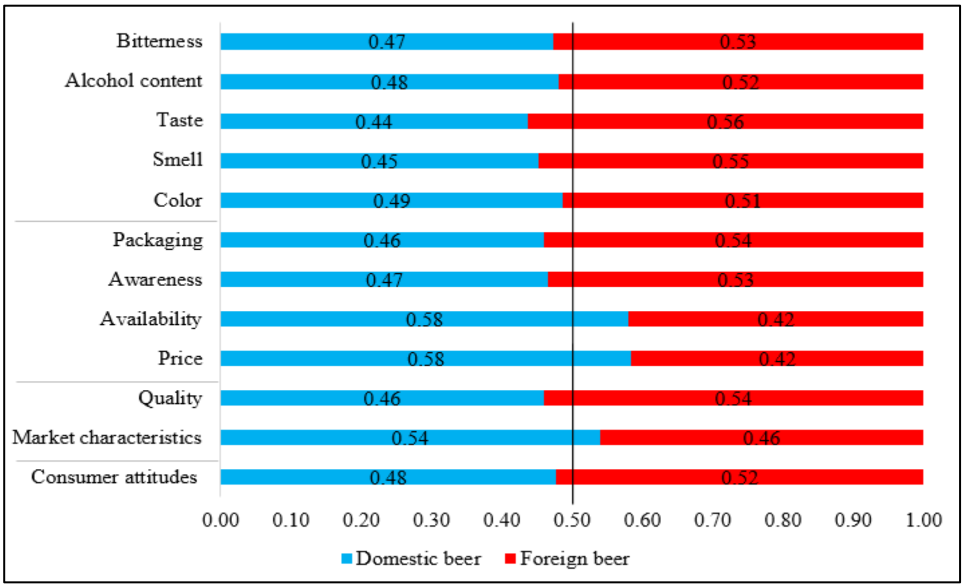

Figure 5. Evaluated alternatives

Source: *Authors' calculation*.

Moving from the bottom of the compared sub-criteria to the top, it is possible to reach the generally most important indicator that, based on self-determined weightings, consumers will choose foreign beers (F-Foreign 52%) rather than domestic ones (D-Domestic 48%). This statement was confirmed earlier by Lekše, (2021), who concluded that older beer consumers tend to exhibit higher ethnocentric tendencies. When it comes to the market characteristics of domestic and imported beers, domestic beers (D 54%) are rated better than imported beers (F 46%), however, when it comes to quality, imported beers are rated better (F 54%) compared to domestic (D 46%). When it comes to sub-criteria, domestic beers are rated better only in terms of Availability and Price (D 58% : F 42% for each comparison). On the basis of all other sub-criteria, imported beers are rated better, with the most dominant difference being evident in Taste (F 56% : D 44%), followed by Smell (F 55% : D 45%), Packaging (F 54% : D 46%), Awareness and Bitterness (F 53% : D 46%), Alcohol content (F 52% : D 48%) and Color (F 51% : D 49%). These results are consistent with the research of Jalić et al. (2021) related to domestic Nektar beer, where the Taste, Smell and Bitterness variables were rated lower also.

Conclusion

The research conducted on a sample of 25 respondents of the Z generation, using the Expert Choice program and the application of the Analytical Hierarchical Process, leads to the following conclusions about consumer behavior when purchasing, consuming and choosing domestic versus foreign beers in Republika Srpska:

• The collected survey responses are consistent, which means that the answers are not mutually contradictory.

• Beer Quality criteria factors are more important than beer Market characteristics factors with a ratio of 76.6 : 23.4%.

• The most important quality factor of buying beer is the taste of the beer. It is followed by bitterness, alcohol content, smell and color.

• The most important factor of market characteristics is price, then availability and finally packaging and familiarity.

• Based on the determined weightings, and alternatives when purchasing, an affinity for domestic or imported beers was determined.

• Domestic beers were rated better than foreign beers for the market characteristics and the sub-criteria of availability and price, which was certainly expected.

• Foreign beers are rated better based on the quality criteria and sub-criteria of bitterness, alcohol content, taste, smell, color, packaging and familiarity.

• The general conclusion of the research, based on the attitudes and opinions of 25 surveyed beer consumers, and weighted criteria and sub-criteria, is that Generation Z has a little greater affinity for foreign beers compared to domestic beers.

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Associating in Serbian agriculture: cooperatives and clusters

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Abstract

Agriculture is an essential branch of the economy, and ways to improve its contribution to the overall economy should be constantly sought. One of the ways and methods to achieve this is by associating in different forms. The main objective of the paper is to analyse the importance of different connection models and the need for the improvement of clustering in agriculture. However, there are different connection models, for more detailed consideration in this paper, cooperatives and clusters were chosen. The focus of the analysis is on Serbia because there are natural preconditions for the development of agricultural production as an economic activity. The interpretive content analysis of secondary sources was used in this article to achieve the paper's objective. The paper presents a theoretical analysis of the cooperatives and clusters in agriculture. The paper consulted a rich literature review. The research was conducted based on the analysis of available sources of literature. The motive of the research and analysis in this paper is the more profound understanding of the benefits brought by the association in clusters and cooperatives. The results of the analysis showed that cooperatives and farmer organisations can provide added value to agribusiness firms, which is a kind of benefit for everyone. The results of the analysis also showed that the clusters can combine local and global perspectives, which can be crucial for the development of agricultural activity in general, and, consequently, the entire economy. The conclusion could be combined for both types of mergers. By joining either in cooperatives or in clusters, resource strength is increased and synergistic effects are achieved that could not be achieved by individuals as members.

Key words: agriculture, associating, cooperatives, clusters, Serbia.

Introduction

The agricultural sector is of great importance in the economy of Serbia, because, among other things, agriculture has a comparative advantage that could bring benefits to Serbia given the favourable natural conditions. In response to environmental challenges, different organisational models for agricultural production have evolved, as well as different forms of associating, connecting and networking, which is, after all, a modern trend that also applies to agribusiness. The new concept of agricultural policy will be much easier to achieve if the principles of market and entrepreneurial behaviour are accepted in agriculture, both by small and medium-sized enterprises from this sector and by agricultural farms (Aničić et al., 2017). Full use of agricultural potential is possible if small agricultural producers are linked to markets to achieve higher income and other benefits (Zakić et al., 2014, 233).

Ashwini et al. (2020, 225) state that agribusiness incubators catalyse entrepreneurship development by facilitating technology and institutionalised services. Furthermore, Newell et al. (2021) point out that a possible means for accelerating the development of agricultural technologies and advancing the fourth agricultural revolution is through business accelerator and incubator (BAI) programs. Within incubator networks in developing countries, there are traditional intensive incubators, such as incubators for the development of cooperatives of impoverished people, incubators in agriculture along value chains that heal whole communities - villages, cyber parks developing socially necessary and ecological products, incubators for development and improvement of the business environment (Ahmetagić & Harmath, 2008, 38). Introducing social entrepreneurial incubators would solve social problems in society and improve the development of local communities. Applying such entrepreneurial incubation models would solve problems in agriculture and other branches of the economy in Serbia (Ožegović, 2012, 354). The existence of the incubator as a form of connection is inevitable and should certainly be mentioned, but further elaboration would require a different conception of the paper, both in scope and thematically. The subject of overview research in this paper is primarily cooperatives and clusters.

From the perspective of smallholder farmers, membership in cooperatives and farmer organisations offers many benefits and the possibility of inclusion in modern agricultural value chains. The association of individuals creates cooperatives, and in the broadest sense, they are seen as an instrument for realising their individual needs (Simonović et al., 2016). With the involvement of cooperatives and the modern requirements of the modern economy, it is possible to contribute to progress in rural areas and provide an incentive for rural development.

Serbia is essentially an agricultural country, and improving the state of cooperatives is vital for the national economy and rural areas. It can rightly be argued that relatively few clusters related to the agricultural and food sector have been covered in the literature, although Ping and Waldemar (2011) state that the agricultural cluster represents a trend of modern agricultural development. These authors also elaborate that agricultural clusters are of great importance for promoting the growth of the regional economy, improving competitiveness, improving the specialisation of agricultural production and increasing farmers' income. When it comes to the specific contribution of the cluster to the improvement of the competitiveness of rural areas and their constant development, the role in the creation of new jobs and the development of entrepreneurship is particularly important, both in the field of primary agriculture and in other sectors of the rural economy. It should not be overlooked that clusters attract financial resources and new members to rural areas (Mitrović & Mitrović, 2020, 257).

The process of popularising the cluster concept is still present because clusters result in themselves (Bianchi, 2005), eliminating many weaknesses that unconnected members or entities cannot overcome, mainly because more problems are caused by their isolation and not size. Nevertheless, clustering in agriculture in Serbia is not at a satisfactory level of development. The main problem is the lack of awareness among producers about the need for associating to achieve synergistic effects in product placement, more innovative production methods, better organisation and the like (Džanković-Jerebičanin, 2014, 118).

Besides desk research and literature review, a comparative analysis was used with the interpretative method approach. The vital role in creating connections between different sources is found in the interpretive method approach, and such approaches should be held to their standards (Schwartz-Shea & Yanow, 2006). The main data sources are national statistics databases and ministries of agriculture's data. An analysis of numerous respectful studies on related topics was performed, and attitudes about implementing cooperative principles in practice were systematised. Accordingly, the method of analysis and synthesis was applied, i.e. based on the existing secondary data and previous research, certain conclusions were drawn. In addition, the interpretive research method is more flexible and holistic because it implies the liberalisation of traditional content analysis coding rules (Ahuvia, 2001).

The paper is designed to consider two key thematic entities, cooperatives as a form of association in agriculture and clusters, which are also a form of association in agribusiness. In addition, the paper conceptually consists of introductory and concluding considerations, as usual.

The article can be useful to the professional and scientific public interested in this issue.

Agricultural cooperatives as a form of associating - generally observed and with a long tradition in Serbia

According to the International Cooperative Alliance (ICA), a cooperative is an autonomous association of people voluntarily associated with meeting mutual economic, social and cultural needs and aspirations through a joint and democratically controlled enterprise (ICA, 1996). Coltrain et al. (2000) define a cooperative as a business operated primarily to benefit members through marketing transactions and the distribution of earnings from these transactions. According to Vorley et al. (2009), collective action is an important strategy to increase small farmers' participation in emerging modern markets and create sustained commercial flows of high-quality products. Osterberg and Nilsson (2009) point out that it is natural for members to wish that the cooperatives are successful businesses and that they are run in the members' interests. Slightly less than three million cooperatives are operating worldwide, with more than one billion cooperative members (Knežević, 2021, 60).

The cooperative, as a form of agricultural production organisation, is considered one of the best instruments of self-protection for small farmers mainly due to its self-help concept and member’s participation (Stojanović & Rokvić-Knežević, 2021, 226). The environment constantly changes and puts intense pressure on cooperatives to change themselves to survive and become more effective and efficient. Agricultural cooperatives perform entrepreneur functions, and Schoonhoven and Romanelli (2001) underline many new directions that explore the idea that entrepreneurship emerges as a function of collective actions. Due to numerous changes in the business environment in the last few decades, different forms of cooperatives have emerged compared to the traditional model. According to Rokvić and Brković (2020) developed new types of cooperatives have evolved that differ on the issues of open membership, member shares, degree of responsibility, outsourcing opportunities, decision-making, and revenue sharing.

Serbia is a country with a rich cooperative history and tradition (Cooperative Union of Serbia, 2020) and primarily agricultural cooperatives. After some time, other types of cooperatives are formed, not only agricultural ones. Classifications of types of cooperatives may depend on many factors, such as activity, capital, i.e. ownership, etc.

In the first half of the 19th century, Serbia had its first cooperative in Bački Petrovac and the first Serbian union of agricultural cooperatives, with headquarters first in Smederevo and then Belgrade. Due to unfavourable and specific circumstances, the development of cooperatives in Serbia took a changing course. Serbia has resources in rural areas that can significantly develop rural areas, contributing to rural development (Mihajlović & Pejčić, 2005). Through cooperatives, rural areas in Serbia can improve living standards and increase employment.

The cooperative organisation has proven to be a sustainable, accepted and successful form of agricultural production development, a pathway to exit and survive on the market, capital penetration in the activities of small farmers, as well as differentiation and individual growth of some of them (Maričić, 2009). Agricultural cooperatives offer the benefits of collective strength, help increase the income of their members, increase their bargaining power with other participants in the value chain, provide essential services, expand the market for their products as well as achieve a better price for farmers, help them acquire better entrepreneurial, marketing and other skills. A study conducted by Zakić et al. (2013) indicated the awareness of cooperative members that appropriate knowledge and skills are required for various administrative and professional tasks, which implies the need to hire appropriate personnel. In addition to benefits to their members - farmers- cooperatives benefit the local (rural) community and consumers (Rodriguez, 2011; Food and Agriculture Organization, 2012).

Cooperatives and other farmer organisations represent one of the essential mechanisms for the involvement of small farmers because they are formed with the motivation of mutual benefits and expectations of collective action among members (Hong & Sporleder, 2007). However, this does not mean that cooperatives and farmers' organisations will be guaranteed success even though they offer the benefits of collective strength. Many cooperatives and farmers' organisations with economic and market objectives have poor results due to internal factors (Garnewska et al., 2011; Elbehri, 2013).

When it comes to Serbia, according to the data of the Strategy for the Development of Agricultural Cooperatives in the Republic of Serbia (2011), the agricultural cooperative is, according to the indicators for 2010, dominant in the overall cooperatives of Serbia, not only according to the participation in the total number of cooperatives but also according to the number (6,292) and the participation (79.6%) of cooperative workers in agriculture in the total number of employees in all types of cooperatives, as well as the fact that they achieved 59 billion dinars or 86% of the total income of cooperatives in Serbia.

Furthermore, according to the data of the Cooperative Union of Serbia, in 2017, 2,600 cooperatives of all types were registered, of which 1,548 were agricultural. According to the data of the Agency for Economic Registers in the Republic of Serbia, 1,568 agricultural cooperatives are active, of which about 1,100 submit financial reports. Of the total number, a third can be seen as inactive cooperatives.

It is characteristic for the period from 2016 until 2020 that the number of newly formed cooperatives exceeded by 462 the closed ones. The majority are traditional agricultural cooperatives (1,293), while the penetration of others is somewhat slower. From 2015 to 2020, 1100 agricultural cooperatives were formed, according to the data of the Cooperative Union of Serbia. In any case, these indicators show that certain developments exist and that agricultural cooperatives in Serbia have a perspective.

Cluster association in agribusiness as a contribution to the economic development of Serbia

By building network structures and horizontal and vertical connections, the cluster members relativise their shortcomings and emphasise their strengths, thereby achieving market competitiveness. This type of connection enables its members to find a way to business success in mutuality of experience and modernity. The advantage of small companies is their flexibility. However, at the same time, what is an advantage can become a disadvantage. Small companies can adapt faster, which is encouraged by fewer employees, who, on the other hand, can have better interaction and a more direct relationship with the owner. When it comes to a small team, people identify more easily with the company and are more ready to help realise its goals, vision and mission.

On the other hand, this hypersensitivity makes them vulnerable regarding costs, for example. Moreover, small firms are often in a dependent, vassal position related to large companies. In overcoming all these shortcomings in managing individual small and medium enterprises, cluster mergers help. There are different theoretical approaches to clustering. Some emphasise its structure and characteristics, others economic impact and the need for clustering. However, it is certainly indisputable that organisations joined in clusters have better interactions and exchanges of information, knowledge, and experiences, producing synergic effects (Mirčetić et al., 2019).

Cluster development policy must not be based on a single strategy applicable in all situations. The experiences of developed countries have shown that the "one size fits all" approach in the formulation, and implementation of policies and programs is ineffective (Vukotić et al., 2013). Regarding Serbia, however, it is safe to say that the results achieved by implementing policies based on business clusters do not correspond to the expectations and potential for economic development associated with clusters (Pandurević, 2012).

Clusters can be initiators of agricultural development (Bell & Giuliani, 2007; Džanković-Jerebičanin, 2014; Kilelu et al., 2017; Paraušić & Domazet, 2018). All agricultural stakeholders have a chance to depreciate or completely overcome the problems mentioned earlier by using examples of good practices or joining agricultural clusters.

The goal of establishing agro clusters in Serbia can be understood and somewhat generalised through the vision of one of the agro clusters, Agro Cluster of Eastern Serbia: "to establish the preconditions for improving and innovating current agricultural technology, food processing, logistics, agritourism, and building new business capabilities for cluster members." Also, the vision of the agro cluster of Eastern Serbia is to establish its own local and regional markets and to expand them into the European and world markets of agricultural products, quality foods, and rural tourism (Vukotić & Mirčetić, 2020). Paraušić et al. (2007) point out that in Serbia, the formation of agro clusters is based on the aspiration for better positioning in the market, not on the country's assistance.

According to the research conducted by Vukotić and Mirčetić (2020), 41 agricultural clusters were established in Serbia, and according to the regional distribution, most of them are in Belgrade and its surroundings (9), followed by South Bačka (7), and Šumadija and Western Serbia (6), while in other regional parts of Serbia, five or less agricultural clusters were established.

Conclusion

The primary objective of all cooperatives is the welfare of their members by improving their economic, social and cultural position. Cooperatives are essential for the country's economy and contribute to developing rural and underdeveloped areas. The cooperative raises the level and quality of life in the countryside by maintaining and increasing production and employing people in administrative and commercial jobs. Also, the existence of agricultural cooperatives is only justified if it benefits its members and improves overall agricultural production.

At the same time, the intensification of associating is a modern trend, and even though, for example, agricultural cooperatives have a long tradition in Serbia, there is always room and need for improvements and advancements. Serbia is a country with a long cooperative tradition. Support for associating in cooperatives and using their advantages, following the example of countries where cooperatives are developed, is one of the bases of conducting a successful economic policy, and in Serbia, there are all the necessary prerequisites for this.

Cluster networking represents an outstanding contribution to increasing the resource strength of various levels, from logistics, marketing, knowledge transfer, opening new markets, increasing exports, attracting investments and many other activities that give synergistic effects in this exchange. The significant popularisation of clusters with wide application in the developed business world is still actual. It can be rightly argued that relatively little research is

conducted relating to the agricultural and food sectors. However, for the agricultural cluster, this in no way diminishes their importance, and research should undoubtedly be intensified, and their implementation in practice should be strengthened.
The cluster brings together organizations, institutions, and associations dealing with activities in various fields of economy and agriculture of Serbia. Given that clusters represent the most favourable form of integration and inclusion for poorly developed local agricultural holdings, cooperatives, micro, small and medium-sized enterprises, entrepreneurial shops and business associations, it can have wider repercussions in agriculture and the economy in general in Serbia. Ultimately, joining agro clusters in Serbia can affect local and regional development, finding new markets, introducing innovations, better access to financial resources, and introducing quality standards and employment.

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Gender differences in characteristics of quality, loyalty, recognition and association with the brand „Valjevsko pivo"

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Abstract

The aim of this empirical research was to determine gender differences in the subjective perceptions of consumers about the most important variables of the „Valjevsko pivo" brand (quality, loyalty, recognition and associations with the brand) with regard to their socio-demographic characteristics (gender, education and place of residence). The sample of respondents (N = 181) included 134 men and 62 women, ages 18 to 70, average age 46.24±9.71 years. The research instruments are Aaker's brand variables and a survey questionnaire on the socio-demographic characteristics of the respondents. Determined relations between groups in the examined variables were defined by Mann-Whitney (U) and Kruskal-Wallis and (K-W) non-parametric tests at the level of statistical significance ($p \leq .05$, $p \leq .01$). The calculated values of the Cronbach Alpha coefficients of internal reliability show that the variables used had satisfactory psychometric characteristics, and that they are a valid measuring instrument that can be recommended for examining the population in Serbia. Depending on the gender, the results of the U and K-W tests show that men have a higher arithmetic mean of ranks in relation to the perception of the variables quality, loyalty, recognition and association with the brand. Also, significant differences were manifested between social-demographic variables (lowest and highest level of education) and measured Aaker variables. However, the statistical analysis indicates that no statistically significant differences were found for the parameters place of residence and attitudes about the „Valjevsko pivo" brand, because the sums of the ranks are approximately equal. The results obtained in this study point to the importance of learning about statistically relevant differences between respondents in the variables of the „Valjevsko pivo" brand, considering their socio-demographic characteristics.

Key words: Aaker's variables, beer, consumers, men, women

Introduction

After water and tea, beer ranks third in terms of frequency of use among people. 1.8 billion hl of beer is produced in the world (Bowden et al., 2019; Silva et al., 2016). Germany has the largest production in Europe, while the Czech Republic has the highest consumption of beer per capita (Popović, 2022; Tahmassebi and Bani Hani, 2020). In Serbia, "Valjevska pivara" ranks among the largest producers of beer (Domazet et al., 2018), which contributes even more to the importance of the Valjevska alcoholic beverage brand. Research in the field of the beer market and consumer behavior of this consumer product in the Republic of Serbia is very rare. „Valjevsko pivo" is one of the most important brands of the Kolubara district and Western Serbia, with a hundred-year tradition of beer production.

According to the authors (Jukić and Dunković, 2010), the term brand was introduced for the first time in ancient Greece and Egypt, with the aim of protecting the customer from bad purchases. The American Marketing Association (AMA) defines a brand as a name, term, sign, symbol or shape, with the intention of identifying the products or services of one or a group of sellers and differentiating the service of one or a group of sellers from the competition (Nakić, 2022). The basic dimensions of the brand are quality, loyalty, recognition and brand association (Aaker, 1991). Researchers (Craine et al., 2021; Rodriguez-Saavedra et al., 2020; Rossi et al., 2021) believe that the brand has a particularly relevant function for consumers in their choice, greater than private labels. Interest in researching the role of the beer brand, i.e. its quality, loyalty, recognition and associations with the brand among consumers, has been shown by several authors (Santos et al., 2021; Vasas et al., 2021).

Using the example of „Nektar pivo“, Jelić et al. (2021) conduct research on consumer attitudes about the main brand variables (quality, loyalty, familiarity and association with the brand) in Bosnia and Herzegovina and Republika Srpska. On this occasion, the authors conclude that the quality and familiarity of the analyzed beer brand is at a satisfactory level, and that consumer loyalty and association with the brand are average.

In the paper (Bellut and Arendt, 2019), brand loyalty is explained as an intense commitment to the purchase of a particular brand, i.e. the quality of the product to which the sign refers. The aforementioned authors believe that brand loyalty is the result of the interaction of emotions and attachment with repeated purchases, and that loyalty is manifested through repeated purchases, but also forgetting mistakes, recruiting new customers by word of mouth and expressing what they like and what they don't like.

The quality of products and services has always occupied a significant place in human life. The quality of the product from the perspective of the consumer is called "perceived quality", which in the mind of the customer means the perception of the product, tangible and intangible. In his study, Matović (2010) points out that quality is important, but that the brand is not built only on quality, because everyone has a personal perception of the quality of certain products, and he points out that the assessed quality is maximally determined by the individual taste of the consumer. A brand is a guarantor of product quality, which assumes that every subsequent purchase of the same brand will manifest an identical level of quality. This brand variable goes a long way in mitigating risk and meeting higher-level needs.
Loyalty to brands represents the attachment that the customer shows to the brand, and it interacts with consumer behavior in the market, which can be marked by the number of repeated purchases. It is also defined as a state in the customer's mind that a particular product is his first choice (Fayrene and Lee, 2011). Brand loyalty can be presented as a manifestation of the "tacit agreement" between the consumer and the brand's product. It is measured by the probability that consumers will use products belonging to a certain brand in the following period regardless of changes in market circumstances and marketing efforts of competitors offering the same product category (Moisescu and Allen, 2010).

Brand recognition is one of the basic goals of building product quality, which indicates its different dimensions by which consumers recognize the basic characteristics (Shariq, 2018). The mentioned author believes that the brand is in the mind of the consumer and in a mutual relationship with the intensity of the brand and the memory of the customer, which is reflected in his ability to identify the brand of the product in different conditions and circumstances. Brand recognition depends on the manifest perception of the brand symbol in the minds of target consumers, as well as the intensity of presence in the media, propaganda messages and the wider market environment (Šerić, 2009).

The association with the brand as an addition to the quality of the product brand represents all the immeasurable things that can be associated with the brand (Stašić, 2012). According to this author, this component of the brand can condition the performance of an action and the recall of information, ensuring a moment of differentiation, a reason for purchase, a positive attitude and feeling when disseminating information about the product. Researchers (Calvo-Porral and Montes-Solla, 2013) assume that brand associations contain a set of images, ideas, facts or any other element that has created an intense correlation with brand knowledge. They believe that the associative network of memory models, once the consumer develops "relationships" with the brand, slowly forgets associations that may lose intensity over time but never disappear forever.

Considering the insufficient research of these phenomena, as well as the controversial data obtained in earlier studies, the goal of this operationalized research was to examine the statistically significant difference in the characteristics of the brand „Valjevsko pivo" (quality, loyalty, recognition and association with the brand) with regard to socio-demographic variables (education and place of residence). In accordance with the previous theoretical frameworks, empirical results and the goal of this transversal research, two initial hypotheses were defined:

H1 - With regard to gender, men compared to women when evaluating the features of quality, loyalty, recognition and associations of the brand „Valjevsko pivo" have a higher sum of answer ranks, i.e. the higher the scale value.

H2 - It is expected that there is a statistically significant difference between the highest and the lowest level of education of the respondent in the dimensions of the „Valjevsko pivo" brand.

H3 - It is assumed that there is no empirical significance in the sum of the ranks of the respondents' differences in the perception of the variable place of residence and the characteristics of the „Valjevsko pivo" brand.

Since there has been no empirical research dealing with respondents' perception of the characteristics of the „Valjevsko pivo" brand and their socio-demographic variables (education and place of residence), the results of this cross-sectional study can provide useful initial information for future research in this area.

Material and Methods

Respondents and research procedure

The sample included (N = 181) respondents, 134 males and 62 females from the town of Valjevo. The age of the subjects ranged from 18 to 70 years, and their average age was AS = 46.24 years (SD = 9.71). The respondents' place of residence is mostly urban areas (75.08%), 15.26% live in the suburbs, and 9.66% of the surveyed respondents live in the village. The level of education of respondents is in 73% of cases medium, high in 22%, while primary education represents 5% of respondents.

Before starting to fill out the questionnaire, the respondents signed the Informed Consent Form for participation in the research, which was voluntary and anonymous, and it was emphasized to them that they could withdraw from participation at any time. The examination was carried out in groups of up to 20 respondents, and it lasted on average about 20 minutes. Respondents filled out the questionnaire during December 2022. The draft and research procedure were approved by the Ethics Committee of the Serbian Academy of Innovative Sciences in Belgrade.

Measuring instruments

The first segment of the survey method contained the socio-demographic features of the respondents, while the second part of the questionnaire was divided into four segments according to Aaker's defined brand variables (Aaker, 1991). Each of them (perceived quality, loyalty, recognition, as well as the association with the brand „Valjevsko pivo" contains a certain number of characteristics with which respondents assessed the level of agreement. The assessment of the associated characteristics on the brand variables was carried out using a 5-point Likert scale (1 - in completely disagree, 5 - completely agree).Brand quality and loyalty were assessed using eight characteristics, recognizability with four, and brand association with six characteristics.

The reliability of the questionnaire was tested with the Cronbach Alpha coefficient of internal reliability (Cronbach α) which for the examined variables is for quality (α =.89), loyalty (α =.92), recognition (α =..87) and brand association (α = 91), which signals the satisfactory reliability of the used measuring instrument (Tabachnick and Fidell, 2013).

Statistical data processing

In order to achieve the set goals of this research, descriptive statistics methods (arithmetic mean and standard deviation) and inferential statistics methods (Mann-Whitney U test and Kruskal-Wallis test) were used (Alberto et al., 2002). Statistically significant result of the differences of the tests used between the variables is based on the p-value probability level ($p \leq .05$), with a confidence level of 95% or with a 99% confidence interval ($p \leq .01$). Data processing was done using Social Sciences (SPSS) version 22.0 software. The data are presented in tabular form.

Results and Discussion

In Table 1, respondents evaluated the beer quality variable using eight characteristics. The subjective assessment of the respondents about the quality of beer was based to the greatest extent on the organoleptic properties of the beer, i.e. intrinsic features of quality, and to a lesser extent through extrinsic properties, such as packaging.

Table 1. Basic descriptive statistics of quality as a variable of the brand „Valjevsko pivo"

Characteristic	*AS*	*SD*
The smell	3.38	1.09
Taste	3.35	1.20
Bitterness	3.37	1.10
The color	3.95	.95
The pleasure of drinking	3.64	1.18
Alcohol	3.80	1.03
Ratio of quality and price	3.82	1.05
Packaging	3.90	.88

Legend. AS = arithmetic mean; *SD* = standard deviation.

By reviewing the scores of the obtained arithmetic averages, i.e. the highest average results in the cells of the table, it can be seen that among the characteristics of the beer, the respondents perceived its color and packaging the best, while the lowest average results of the respondents were found for the variables taste, bitterness and smell of the beer, which they consider less important . Also, it is visible that the calculated table values of the standard deviation do not deviate statistically significantly from the arithmetic mean, which signals that the respondents manifest the homogeneity of the answers.

Table 2. shows the basic descriptive data of the results of the loyalty variable.

Table 2. Basic descriptive parameters of loyalty as variables of the brand „Valjevsko pivo"

Characteristic	*AS*	*SD*
Of all the beers, I choose „Valjevsko pivo"	2.99	1.50
Homemade	3.82	1.32
Rising prices affect choice	2.53	1.28
Fulfilling expectations	3.96	1.20
Commitment to consumer interest	3.18	1.05
Irreplaceability	2.58	1.35
Trust in the „Valjevsko pivo" brand	3.19	1.10
Preference choice with identical features	3.40	1.36

Legend. AS = arithmetic mean; *SD* = standard deviation.

The respondents achieved the maximum result when it comes to sensitivity to origin. The findings obtained in this cross-sectional study are consistent with research (Kim et al., 2019) which stated that beer consumers give preference to domestic brands of beer, because they believe that the significant characteristics within this variable is giving the first place when choosing over other beers with identical features, trust in this brand and marketing orientation towards consumers. Thus, consumers have manifested price sensitivity and this characteristic is exposed to danger when associated with customer loyalty. However, the authors (Anderson et al., 2020; Bartram et al., 2017; Gregurec, 2017) conclude that the price is more important to consumers who use beer more often than to those who rarely consume this product.

Recognition as a variable of the brand „Valjevsko pivo" is analyzed in Table 3.

Table 3. Arithmetic means and standard deviations of the recognizability variable

Characteristic	*AS*	*SD*
Media visibility	4.01	1.10
Is the most famous beer	4.23	.89
Is beer always the most desirable	3.59	1.20
Is the most famous brand	4.10	.88

Legend. AS = arithmetic mean; *SD* = standard deviation.

Respondents rated the characteristics of the most famous beer and the most famous brand the best for the variable recognition. This is also logical since „Valjevska pivara" has a long historical tradition of 162 years. The attribute media visibility of beer also shows a high level, with answers clustering around 4 on the Likert scale. At the same time, the respondents judged that this beer is the most desirable on the domestic market. According to research (Bušić, 2022; Gallage et al., 2020), the reasons for using domestic beer are greater accessibility, lower price, stimulus to the domestic economy, while some believe that domestic beer is the best, and better quality and taste of domestic beer.

Table 4 shows the basic static parameters of associations as variables of the brand „Valjevsko pivo".

Table 4. Basic descriptive statistics of associations as variables of the brand „Valjevsko pivo"

Characteristic	*AS*	*SD*
Happy moments and events	3.25	1.29
Connecting in a quality consciousness	3.01	1.23
Connecting in awareness of significance	2.73	1.30
Connecting in the awareness of history and tradition	3.55	1.24
Association with the introduction of a new product	3.18	1.12
Association for the selection of an excellent product	3.37	1.25

Legend. AS = arithmetic mean; *SD* = standard deviation.

The highest arithmetic mean of all the characteristics of the association variable of the brand „Valjevsko pivo", which is expected considering its history and tradition of „Valjevsko pivo". The data obtained correlates with research (Brown and Murphy, 2020; McGreen et al., 2022; Neelakantan et al., 2022) in which it was determined that beer is a characteristic worker's drink that is dominantly associated with socializing, a great atmosphere and relaxation. The connection in the awareness of the quality of „Valjevsko pivo" is a below-average rated characteristic, while the association with the connection in the awareness of the importance is the worst rated feature of this variable. This is interesting information since the quality variable, ie. its individual elements are positively perceived by the respondents.

In Table 5, the respondents evaluated the total values of the characteristics of the investigated variables of the brand „Valjevsko pivo".

Table 5. Arithmetic means and standard deviations of measured variables Brand „Valjevsko pivo"

Characteristic	*AS*	*SD*
Quality	3.76	.93
Loyalty	3.05	1.10
Recognizability	4.08	.90
Associations	2.99	1.12

Legend. AS = arithmetic mean; *SD* = standard deviation.

Inspection of the given tabular data shows that recognition is the best-estimated variable of the „Valjevsko pivo" brand. Also, the variable quality of „Valjevsko pivo" was highly evaluated. The attributes of brand association and loyalty are less evaluated compared to the previous two variables, showing a satisfactory average level. The maximum variation in the responses to brand associations is visible, which has been confirmed in studies (Müller et al., 2021; Silva et al., 2021; Suthovski et al., 2021). The mentioned authors established that the following factors contribute the most to consumer preferences when choosing beer: taste, quality and brand, as well as that there is no relevant consumer loyalty towards the same beer producer.

Table 6 shows the gender differences among the respondents in the socio-demographic variables and variables of the „Valjevsko pivo" brand.

Table 6. Mann-Whitney U test and Kruskal-Wallis test values

Sum of ranks U					
Characteristic	Rank Quality	Quality	Loyalty	Recognition	Associations
Gender	Men	187,26	180,74	179,45	175,80
	Women	198,76	209,66	199,05	224,69
	p	.05	.01	.05	.01
Education	Basic	29.06	30.65	33.42	30.01
	Medium	20.17	20.56	20.98	20.64
	High	20.06	20.11	20.73	18.34
	p	.05	.01	.05	.01
Place of living	City	20.76	20.53	19.24	20.03
	The countryside	20.09	19.67	18.53	20.73
	Suburb	20.02	20.05	25.90	23.45
	p	.07	.06	.08	.07

Legend. * $p \leq .05$; ** $p \leq .01$.

The results of the tests of the sum of the ranks of the non-parametric MW U and K-W tests show that the respondents differ significantly, with the reliability of 95% or 99%, that is, at the level of significance ($p \leq .05$, $p \leq .01$). This means, with regard to gender, that men compared to women when evaluating the variables quality, loyalty, recognition and associations as variables of the brand „Valjevsko pivo" have a higher arithmetic mean of ranks, i.e. the higher the scale value. Considering the obtained results, the H1 hypothesis is confirmed, which assumed that men compared to women have a higher sum of answer ranks, i.e. a higher value of the scale when assessing the characteristics of quality, loyalty, recognition and associations. Also, based on the parameters of the test statistics obtained in this transversal study, it is evident that the socio-demographic characteristics of the respondents, i.e. education has an influence on their attitudes about the „Valjevsko pivo" brand. Therefore, with a risk of 5% or 1%, a statistically significant difference can be observed between the attitudes of respondents with the lowest and highest levels of education and the variables quality, loyalty, recognition and association with the examined brand. The obtained findings confirmed the tested hypothesis H2, i.e. the expectation that there is a statistically significant difference between the highest and the lowest level of education of the respondents in the dimensions of the „Valjevsko pivo" brand. On the other hand, for the parameters place of residence and attitudes about the brand „Valjevsko pivo", at the level of significance ($p \leq .05$) no significant differences in the ranks within the analyzed variables were found, since the sums of the ranks are approximately equal. This is proof that with the probability of a random result greater than 5%, the initial hypothesis H3 can be accepted in the conducted research, i.e. the expectation that there is no statistically significant empirical difference between respondents in the perception (variable place of residence) and the characteristics of the „Valjevsko pivo" brand.

Although satisfactory psychometric characteristics of the used measuring instruments were evaluated in this cross-sectional study, it is important to note several methodological shortcomings, which to a certain extent could have conditioned the obtained results and which should be analyzed when interpreting the obtained results. The first methodological limit is a small number of measured variables, where the sample included relatively few respondents in a limited geographical area, which is insufficiently representative, and the results cannot be generalized to the general population in Serbia. Secondly, the sample of respondents used exclusively the method of self-assessment of all variables, which is why it can be assumed that socially desirable answers are given, as well as the existence of methodological variance and problems with the perception of one's own behavior. The third limitation refers to measures of variability and testing methods of self-assessment of differences between two arithmetic means that did not give a clear answer about the causal relationship between the analyzed variables. Future research should overcome the aforementioned methodological limitations, and instead of a transversal design that tests respondents in a given time period (at one point in time), be longitudinal and include a larger representative sample of respondents over a longer period of time, with the introduction of a new battery of variables, such as personality dimensions, and motivations, which influence the variables of the „Valjevsko pivo" brand (quality, loyalty, recognition and associations with the brand). However, despite the mentioned methodological shortcomings, this cross-sectional empirical study provided useful results and a relevant basis for further research, and therefore has important implications for practice.

Conclusion

The conducted empirical research examined gender differences in individual consumer perceptions of the most relevant variables of the „Valjevsko pivo" brand (quality, loyalty, recognition and associations with the brand). Cronbach's alpha reliability coefficients (Cronbach α) defined a satisfactory level of internal consistency for all applied variables, which points to their validity and reliable application in the Serbian-speaking population as well. Non-parametric tests of inferential statistics (Mann-Whitney U test and Kruskal-Wallis), with statistical significance of $p \leq .05$, $p \leq .01$, on our sample suggest the following: 1) male gender with regard to female gender when observing Aaker's variables manifests a greater arithmetic mean of ranks; 2) there is a statistically significant difference between socio-demographic characteristics (lowest and highest level of education) and the investigated Aaker variable; 3) the absence of a significant difference between the variables place of residence and attitudes about the brand „Valjevsko pivo".

Given that in the Serbian-speaking area there is a lack of quantitative research on identifying respondents' self-assessments about the characteristics of the „Valjevsko pivo" brand and their socio-demographic characteristics (education and place of residence), the results obtained in this transversal study provide important initial knowledge for a better understanding of this problems on the population in the Republic of Serbia.

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Полне разлике у карактеристикама квалитета, лојалности, препознатљивости и асоцијацији на бренд „Ваљевско пиво”

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Апстракт

Циљ овог емпиријског истраживања био је утврдити полне разлике у субјективним перцепцијама потрошача о најважнијим варијаблама бренда „Ваљевско пиво“ (квалитет, лојалност, препознатљивост и асоцијације на бренд) с обзиром на њихове социо-демографске карактеристике (пол, образовање и место становања). Узорак испитаника (H = 181) обухватио је 134 мушкараца и 62 особа женског пола, старости од 18 до 70 година, просечне старости 46,24±9,71 година. Инструменте истраживања представљају Аакерове варијабле бренда и анкетни упитник о социо-демографским обележјима испитаника. Утврђени односи између група код испитиваних варијабли дефинисани су Ман-Витни (U) и Крускал-Валис и (K-W) непараметријским тестовима на нивоу статистичке значајности ($p \leq .05$, $p \leq .01$). Израчунате вредности Кромбах Алфа коефицијената интерне поузданости показују да су коришћене варијабле имале задовољавајуће психометријске карактеристике, те да су валидан мерни инструмент који се може препоручити и за испитивање популације у Србији. У зависности од пола, резултати тестова *U и K-W* показују да мушкарци у односу на жене код перцепције варијабли квалитет, лојалност, препознатљивост и асоцијације на бренд имају вишу аритметичку средину рангова. Такође, манифестоване су сигнификантне разлике између социјално-демографских варијабли (најнижи и највиши степен образовања) и мерених Аакерових варијабли. Међутим, статистичка анализа указује да код параметара место становања и ставова о бренду „Ваљевско пиво“ нису установљене статистички значајне разлике, јер су суме рангова апроксимативно једнаке. Добијени резултати у овој студији упућују на значај сазнања о статистички релевантним разликама између испитаника/ца у варијаблама бренда „Ваљевско пиво*”* с обзиром на њихове социо-едемографске карактеристике.

Кључне ријечи: Акерове варијабле, пиво, потрошачи, мушкарци, жене

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Variability of mastitis occurrence in dairy Simmentals due to recording time

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Abstract

The aim of this research was to determine the mastitis occurrence in dairy Simmental cows regarding the year, month and season of milk recording. Therefore, test-day records were collected during regular milk recording on dairy cattle farms in the period from January / 2004 – December / 2022 were analysed. After logical control, the processed dataset contained 4,922,751 test-day records. The obtained results indicated the effect of the time of recording (year, month, and season) on mastitis occurrence in dairy Simmental cows. The highest occurrence of cows with mastitis was determined in the year 2020 (25.76%); in August (21.89%) and during the Summer season (20.89%). During the analysed period (2005 – 2022), 35% of cows experienced health problems caused by mastitis occurrence. Therefore, it is necessary to put into practice a system for monitoring and prevention of mastitis occurrence in order to ensure successful management and minimize the environmental impact of dairy farms.

Key words: dairy Simmentals, test-day records, mastitis, occurrence

Introduction

Mastitis represents the inflammation of the mammary gland or udder and could occur in an acute or chronic state, and subclinical or clinical phase. According, to many studies (Seegers et al., 2003; Petrovski et al., 2006; Gráff and Mikó, 2015; Ibrahim, 2017) mastitis is one of the most frequent and most expensive disease on dairy cattle farms. The prevalence of mastitis in dairy cattle herds varies from 20% (Hasan et al., 2018) to more than 70% (Sayeed et al., 2020) and generates significant losses for the farm due to decreased milk production, increased treatment costs, declined udder functionality and finally decreased productive life of the infected dairy cow. Since subclinical mastitis is difficult to determine, it causes higher costs for dairy farms in comparison to the clinical forms that could be easily detected and treated (Gráff and Mikó, 2015). For dairy farmers, it is of vital importance to be able to detect any disorder or disease as earlier as possible so that they could act properly and timely in order to ensure that milk production is sustainable from an economic and environmental aspect. Early detection of possible problems and accurate monitoring of dairy herds could be obtained by the application of various sensors and systems of precision dairy cattle technologies. Bewley (2010) emphasised that precision dairy cattle technologies enable the maximisation of the genetic potential of animals at the individual level as well as the minimization of the use of medicaments by using preventive health measures.

Milk recording as well as the analysis and use of milk recording data for various evaluations and predictions represent one of the precision dairy technologies. These data could be used as an excellent tool for the early detection of various disorders and diseases at the individual animal level. Furthermore, early detection and prevention of further development of a particular disorder or disease of the animal enable the reduction of treatment and production, then optimization of management on a dairy farm as well as more climate-friendly milk production.

The occurrence of mastitis harms the udder tissue and decreases the synthetic capacity of secretory cellular enzyme systems, resulting in reduced lactose biosynthesis (Kitchen, 1981) as well as elevated somatic cell count, SCC (Botton et al., 2019). Therefore, both milk parameters, lactose content and somatic cell count could be an indicator of inflammation of the mammary glands of lactating cows (Pyorala, 2003; Ivanov et al., 2016).

Considering the increasing importance of preventing the occurrence of various disorders and diseases on dairy farms, ensuring antibiotic-free farming and ensuring environmentally sustainable systems of animal production, this research aimed to determine the occurrence of mastitis in the population of dairy cows of the Simmental breed depending on the year, month and season of milk recording based on milk recording data.

Material and Methods

For statistical analysis, test-day records of dairy Simmental cows collected in the period from January, 2004 – December, 2022 were used. Data were collected during regular milk recording on dairy cattle farms situated in Croatia. Milk recording in Croatia has been performed accordingly to the alternative milk recording method (AT4 / BT4), which implies measuring milk yield and sampling of milk of each cow in lactation during evening or morning milking every four weeks. Furthermore, milk recording was performed by field officers of the Croatian Agency for Agriculture and Food (AT4) or trained farm worker (BT4). The samples of milk were analysed in the Central Laboratory for Milk Quality Control in accordance with accredited laboratory methods; infrared spectrophotometry for determination of lactose content (ISO 9622:2013) and fluoro-optoelectronic method for somatic cell counting (ISO 13366-2:2006/AC:2007). The following equipment was used in the laboratory: Milcoscan FT6000 (Foss, Denmark) for determination of milk components, and Fossomatic FC5000 (Foss, Denmark) for somatic cell count. Logical control of the dataset implied the following thresholds: stage of lactation in (> 5 days and <500 days), parity from 1 to 9, age at first calving in (> 21 and < 36 months).

Furthermore, all test-day records with missing information regarding parity, breed and missing or senseless values of daily milk traits in line with ICAR standards (ICAR, 2017) were deleted from the dataset. Acordingly to the parity, test-day records were divided in four classes: 1, 2, 3, and 4+. After logical control, the processed dataset contained 4,922,751 test-day records. The basic statistical parameters of analysed traits regarding parity are presented in Table 1.

Table 1. Basic statistical parameters of analysed traits

Parity	**Daily milk yield, kg**				**SCC log**			
	N	**Mean**	**SD**	**CV**	**N**	**Mean**	**SD**	**CV**
1	1204187	16.5	5.48	33.266	1203876	6.4	1.97	30.918
2	1044570	17.4	6.42	36.814	1044313	6.7	2.05	30.791
3	850043	17.8	6.58	36.996	849830	6.9	2.08	30.170
4+	1823951	16.8	6.21	36.847	1823521	7.3	2.13	29.236
Total	4922751	17.0	6.17	36.198	4921540	6.9	2.10	30.582

Furthermore, concerning daily somatic cell count (SCC), cows were divided into three classes: cows with mastitis (SCC > 400,000/ml), healthy cows (SCC < 200,000/ml), and cows at mastitis risk (SCC = 200,000 - 400,000/ml). The occurrence of mastitis in the population of dairy Simmental cows was determined concerning the year (from 2005 to 2022), month (from January to December) and season (Spring, Summer, Autumn, and Winter) of milk recording. For logical control and statistical analysis of data SAS software (SAS Institute Inc., 2019) was used.

Results and Discussion

The occurrence of mastitis in the population of dairy Simmental cows regarding the year of milk recording (2005 to 2022) is presented in Table 2. The lowest percentage of cows with mastitis was determined in 2013 (17.58%), while the highest occurrence was observed in 2020 (25.76%). Furthermore, the highest occurrence of animals at risk (15.99%) was observed in 2019, while the lowest occurrence of animals at risk in the total population under the milk recording was observed in the year 2012.

Table 2. The occurrence of mastitis in population of Simmental cows regarding the year of milk recording (2005 to 2022)

Recording year	Mastitis score regarding the somatic cell count (000/ml)						Total	
	Mastitis > 400		Normal 400 - 200		At risk < 200			
	N	%	N	%	N	%	N	%
2005	17787	19.99	57815	64.99	13361	15.02	88963	100.00
2006	66238	19.32	228091	66.52	48547	14.16	342876	100.00
2007	67465	18.31	249946	67.82	51140	13.88	368551	100.00
2008	73565	18.40	270769	67.73	55429	13.87	399763	100.00
2009	72245	18.86	256979	67.09	53808	14.05	383032	100.00
2010	49339	19.01	173423	66.80	36845	14.19	259607	100.00
2011	50679	18.44	185686	67.56	38471	14.00	274836	100.00
2012	57141	18.29	211943	67.85	43280	13.86	312364	100.00
2013	52554	17.58	203743	68.16	42605	14.25	298902	100.00
2014	53131	18.05	199043	67.64	42104	14.31	294278	100.00
2015	54247	18.61	194884	66.84	42429	14.55	291560	100.00
2016	52231	18.53	189778	67.34	39820	14.13	281829	100.00
2017	50883	19.24	175282	66.28	38306	14.48	264471	100.00
2018	59361	22.81	161300	61.99	39527	15.19	260188	100.00
2019	63070	25.42	145400	58.59	39687	15.99	248157	100.00
2020	54325	25.76	123318	58.47	33255	15.77	210898	100.00
2021	57451	25.04	136561	59.51	35465	15.45	229477	100.00
2022	27847	23.97	71185	61.28	17139	14.75	116171	100.00
Total	979560	19.89	3235147	65.68	711218	14.44	4925925	100.00

The variability in the occurrence of mastitis in dairy Simmentals due to the month of milk recording is presented in Table 3. The highest occurrence of cows with mastitis in an amount higher than 21% was determined in summer months with the highest value in August (21.89%), while the lowest occurrence was observed in April (17.62%). Similarly, the lowest percentage of animals at risk (13.63%) was observed in April, while the highest occurrence of animals at risk in the total population under the milk recording was observed in December.

Table 3. The occurrence of mastitis in population of Simmental cows regarding the month of milk recording (January – December)

Recording month	Mastitis score regarding the somatic cell count (000/ml)						Total	
	Mastitis > 400		Normal 400 - 200		At risk < 200			
	N	%	N	%	N	%	N	%
1	85414	19.78	281924	65.30	64380	14.91	431718	100.00
2	85856	19.47	289720	65.72	65285	14.81	440861	100.00
3	86297	18.28	318329	67.44	67365	14.27	471991	100.00
4	70515	17.62	275222	68.75	54570	13.63	400307	100.00
5	75073	18.61	273283	67.73	55145	13.67	403501	100.00
6	69212	19.58	235194	66.55	49020	13.87	353426	100.00
7	79688	21.23	242339	64.55	53405	14.22	375432	100.00
8	72779	21.89	211739	63.68	47991	14.43	332509	100.00
9	96907	21.75	283947	63.73	64662	14.51	445516	100.00
10	90577	20.35	289514	65.05	65000	14.60	445091	100.00
11	89725	20.38	284504	64.62	66038	15.00	440267	100.00
12	77517	20.12	249432	64.74	58357	15.15	385306	100.00
Total	979560	19.89	3235147	65.68	711218	14.44	4925925	100.00

The variability in the percentage of Simmental cows with potential mastitis regarding the season of milk recording (Spring to Winter) is shown in Table 4. The highest percentage of healthy cows without mastitis-related problems in an amount of 67.94% was determined in the Spring season, while the highest occurrence of cows with mastitis was during the Summer season (20.89%). Furthermore, the highest percentage of animals at risk (14.95%) was observed in winter.

The fact that in the period from 2005 to 2022, only 65% of cows did not have health problems caused by mastitis indicates an extremely large and expensive problem for dairy farms due to the occurrence of mastitis. Therefore, it is necessary to enable well-organized monitoring and prevention of the mastitis occurrence and ensure the successful management of dairy farms by minimizing the impact on the environment in aim of production of healthy and high-quality milk for the market.

Table 4. The occurrence of mastitis in population of Simmental cows regarding the season of milk recording (Spring – Winter)

Season	Mastitis score regarding the somatic cell count (000/ml)						Total	
	Mastitis > 400		Normal 400 - 200		At risk < 200			
	N	%	N	%	N	%	N	%
Spring	231885	18.18	866834	67.94	177080	13.88	1275799	100.00
Summer	221679	20.89	689272	64.94	150416	14.17	1061367	100.00
Autumn	277209	20.83	857965	64.47	195700	14.70	1330874	100.00
Winter	248787	19.78	821076	65.27	188022	14.95	1257885	100.00
Total	979560	19.89	3235147	65.68	711218	14.44	4925925	100.00

The obtained results in this analysis indicate the effect of the time of recording (year, month, and season) on mastitis occurrence in dairy Simmental cows. Similarly, Nóbrega and Langoni (2011) determined a higher incidence of cows with intramammary infections in the rainy season in comparison to the dry season. Changes in the composition of milk, somatic cell counts (SCC) and consequently mastitis occurrence due to heat stress environment during the summer season were reported by Gantner et al. (2011). Furthermore, Weber et al. (2020) confirmed the effect of the season on the quality and quantity of milk, indicating better quality during the winter and spring seasons, while the quality decreased in the hotter months of the summer and autumn seasons.

Conclusion

The results obtained in this research indicate the existence of the variability of mastitis occurrence in dairy Simmental cows due to the time of milk recording (year, month, and season). The highest occurrence of cows with mastitis was determined in the year 2020; in August and during the Summer season. Furthermore, during the analysed period (2005 to 2022), 65% of the dairy Simmental population was healthy, while 35% of cows experienced health problems caused by the occurrence of mastitis. Therefore, it is necessary to put a system for monitoring and prevention of mastitis occurrence into practice in order to optimize management and minimize the environmental impact of dairy farms.

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Animal welfare in cattle breeding

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Abstract

The paper aimed to define the basic elements and the importance of welfare as well as the subsidy system for welfare in animal production in Croatia. The importance of animal welfare is reflected in the commitment of various organizations and policies that deal with this area. Therefore, it's not surprising that animal welfare is the subject of agricultural policy in the European Union which through a system of subsidies in agriculture strives to increase the welfare of animals. The importance of welfare is confirmed by International Organization for the Control of domestic animal productivity - ICAR, which develops specific models and calculates the welfare index of individual farms, and based on that data is concluded that greater animal welfare leads to higher milk yields of cows, and higher satisfaction and health of the farmers, but also a better financial result of the farms.

Key words: welfare, welfare assessment, dairy cows, legislation

Introduction

Animal welfare was always important to farm owners because the quality of life of both farmers and their families depended on the condition of the animals and the benefits they receive from them. Therefore, it's not surprising besides of farm owners, veterinarians, traders, numerous consultants, organizations and agencies implement laws that were created and passed based on of research, aiming for the protection and animal welfare.

Records from early 1965 indicate that animal welfare was emphasised by Prof. Brambell from Great Britain (Brambell, 1965) proposed five „fundamental“ freedoms that are supposed to be ensured: that animals can lie down, stand up and move, stretch and care for itself regardless of its purpose or manner of holding. Furthermore, freedoms were revised in 1979 (Anonym, 1979) and refer to:

1. Freedom from hunger, thirst and poor nutrition through direct access to fresh food and water,
2. Freedom from discomfort by providing an appropriate environment (accommodation) including law and comfortable resting area,
3. Freedom from pain, injuries and diseases through their prevention or rapid diagnosis and treatment,
4. Freedom to express the behaviour characteristic of species by ensuring sufficient space, appropriate housing and social contact with animals of the same species,
5. Freedom from fear, and stress by ensuring conditions that don't cause mental suffering.

International organizations for animal welfare of animals in the world

Today, animal welfare is based on the development and setting of certain standards, educating people who deal with animals, increasing and strengthening the capacity of farms, monitoring the implementation of adopted standards and policies, and communicating with governments and organizations that deal with animal welfare. One of the oldest organizations is the IDF, which was founded way back in 1903 in Brussels, Belgium at the 1st International Dairy Congress to feed the world with safe and sustainable dairy products, developing standards that have a basis in science to increase trust in dairy products and production (IDF, 2021). The IDF cooperates with other international organizations such as FAS, WHO, OIE and ISO to harmonize standards concerning the milk and milk products sector at the global level. Guided by this, back in 2008 a project group of experts and members of the IDF standing committees for farm management and animal health and representatives of the World Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) issued a guide to the welfare of animals in milk production. They identified five key areas of action in the development and implementation of a quality management system for the welfare of dairy animals. They state that these are:

1. Good storage,
2. Proper nutrition,
3. Adequate physical environment,
4. Responsible breeding practices and
5. Robust health management (IDF, 2021).

Furthermore, the importance of compliance in all areas is crucial because only milk from healthy and well-cared-for animals could ensure food security for millions of people around the world.

There are many organizations in the world that deal with the area of welfare, and one of the most famous is the World Organization for Animal Protection (OIE). OIE in cooperation with ISO (The World Federation of National Standards Bodies) developed the ISO/TS 34700 standards for animal welfare, which are based on ethical principles and serve to standardize the rules and behaviour towards animals in all their stages of development (Tranchard, 2016). It benefits everyone, from producers, sellers, processors, and buyers, and can also be used by competent bodies that deal with the implementation of animal welfare standards in the public or private sector that meet the conditions of OIE TAHC. In that way, they provide support in the practice of animal welfare. The OIE Guiding Principles on Animal Welfare also mention „Five Freedoms", which outline five aspects of animal welfare under human control. Furthermore, accordingly to Tranchard (2016), the food industry is taking more action to better implement animal welfare management. The new ISO technical specification ISO/TS 34700:2016, Animal welfare management - General requirements and guidelines for organizations in the food supply chain will help the food and feed industry to develop an animal welfare plan that is aligned with the principles of the World Organization and Animal Health (OIE), and Terrestrial Animal Health Code (TAHC) as well as that ensure the welfare of domestic animals throughout the supply chain. ISO/TS 34700:2016 applies to terrestrial animals that are bred or kept for the production of food or animal feed in certain areas that are excluded from the standard. Furthermore, Tranchard (2016) stated that the OIE TAHC Animal Welfare Introductory Recommendation Document Facilitating the Implementation of Animal Welfare Standards in Chapter 7 elaborates on the topics:

- Transportation of animals by sea,
- Land transport of animals,
- Transport of animals by air,
- Slaughter of animals,
- Animal welfare and fattening cattle production systems,
- Animal welfare and broiler production systems,
- Animal welfare and dairy cow production systems.

Picture 1. shows dairy cows in the pasture that implies breeding following the recommended animal welfare standards.

Picture 1. Dairy cows at the pasture (photo: Mikić, 2022)

Animal Welfare in Croatia

With the adoption of the Animal Protection Act which is in line with the legal acts of the EU, animal welfare is becoming an increasingly researched topic in Croatia. As stated in Animal Protection Act (NN 102/ 17, 32/19), this Law prescribes the responsibilities and obligations of natural and legal persons for the protection of animals during use, which includes the protection of their lives, health and well-being, the manner of handling animals, the conditions necessary for the protection of animals during keeping, breeding, performing procedures on animals, killing, transportation, using animals for scientific purposes, keeping animals in zoos, in circuses and long shows, when selling pets and dealing with abandoned and lost animals, inspection supervision and misdemeanour provisions. Respecting the laws of the Republic of Croatia through the Agency for Payments in Agriculture and in line with the Common Agricultural Policy (CAP), support measures for farmers are implemented through the Rural development programs of the Republic of Croatia, and from May 2018, it starts through the measure M14 - animal welfare (MP, 2023). Furthermore, M14 includes the application of high standards of animal welfare that go beyond current legislation or common practice in the following areas:

- Provision of water, food and care for animals following their natural needs,
- Ensuring housing conditions in terms of enough space, adequate litter and natural light,
- Access to the open air,
- Avoiding mutilation and/or castration of animals, and if necessary the use of anaesthetics, analgesics and anti-inflammatory drugs or immune castration (MP, 2023).

M14 contains five types of operations. Payment for animal welfare in cattle, pig, poultry, goat and sheep breeding (MP, 2023) and we will deal with animal welfare in cattle under the dairy cow category. M14 through operation 14.1.1.: the welfare of animals in cattle breeding provides support in the animal categories of dairy cows, fattening steers and calves where it is necessary to have 4 cows, 7 steers for fattening and 10 calves to be able to receive the support. Well-being on the farm includes three areas of well-being:

- improved nutrition,
- housing conditions and
- access to the open.

Furthermore, accordingly to measure M14 - animal welfare (MP, 2023), in the case of improved nutrition, subsidies can only be realized if it is combined with another area of well-being or improved housing conditions or access to the outdoors. For the improved nutrition measure itself, it is important to comply with two requirements, namely the nutrition plan and the control of mould and mycotoxins. The feeding plan is important not only from the financial side-economic losses but also due to the health condition of the animals because only proper nutrition can ensure good health, growth and maximum protection of domestic animals and also prevent food contamination caused by mycotoxins which lead to animal diseases. This measure contains the creation of a feeding plan based on the day, month and year in accordance with the number of heads and the expected production. The counselling service must give its consent to the prepared feeding plan while control of mould and mycotoxins is analysed for aflatoxin B1 by a certified laboratory and the findings are submitted to the Agency at least by the end of the year.

Also, in the category of improved housing conditions, it's important to fulfil one of two requirements (MP, 2023). The first is to increase floor area which implies an increase in the net floor area that doesn't include obstacles, feeders or drinking troughs by at least 10% compared to prescribed standards. Second is the enrichment of beds in such a way that mattresses are placed on beds for dairy cows or rubber mats for fattening beef and calves at a minimum of 25% in the barn. The most commonly used bed enrichment with litter is at least 3 kg per day for dairy cows or beef cattle and at least 1.5 kg per day for calves. These procedures are applied to achieve greater comfort for the animals while resting and easier getting up and lying down.

Picture 2. Bedding of dairy cows (photo: Mikić, 2022)

The third category of animal welfare subsidies is access to the open where at least one of two requirements must also be met (MP, 2023). The first is to keep livestock on pasture at least 120 days per year meeting all prescribed rules of animal welfare and zootechnical keeping conditions, and it only applies to dairy cows. The second requirement is keeping the animals on the outlet for at least 150 days a year, so the animals are released on open outlets in or near the barn where the animals have free access and are allowed to move freely following the size of the floor area prescribed by law. Depending on which subsidies farmers apply for, it is necessary to complete education in the field of animal welfare for at least 4 hours a year and to keep an appropriate record form in which all required data are regularly entered to be able to monitor and control the implementation of prescribed obligations. The duration of these measures is the calendar year, from January 1. to December 31.

World research on welfare

Canadian network Lactanet for dairy excellence conducted research on the topic of animal welfare, to develop a dairy management tool that enables the assessment and monitoring of welfare status on dairy farms based on DHI data (Luntz and Krogmeier, 2019). They wanted to show the well-being of animals through the generation of a well-being index, respectively welfare of the herd. Welfare was observed through four focus domains:

1. Longevity
2. Nutrition and production

3. Calves and youth
4. Health and reproduction.

In that way, they analyzed a model which evaluates animal welfare through several areas and indicators such as longevity (lactation numbers, excreted and dead cows), nutrition and production (urea in milk, management, cows in transitions and cow production), calves and youth (mortality of calf and age of heifers at calving) and health production (abortion, beta-hydroxybutyrate level, number of somatic cells in milk, ratio of protein and fat in milk, lameness and cow injuries) etc. (Luntz and Krogmeier, 2019). Based on that research it could be emphasised that there are a lot of factors that influence on milk yield of cows, most important are genetics, nutrition which is related to diet and quality of food, conditions in which they live, the way animals are treated, number of milking on daily basis, number of lactations, age, health condition, and method of treatment. Furthermore, Luntz and Krogmeier (2019) conducted research in Germany (Bavaria) in the period from January to October 2017, on 29.636 first parity Simmentals reared on 6.068 different farms. They scored animal welfare accordingly to three criteria – skin damage, pollution/contamination, and movement which they graded on a scale from 1 to 5. A low grade is the worst, while A high grade is the best. The scale of evaluated traits accordingly to Luntz and Krogmeier (2019) is presented in Table 1.

Table 1. The scale of evaluated traits (1 - 5; Luntz and Krogmeier, 2019)

Score	Skin	Cleaness	Mobility
1.	Very noticeable changes in the rear legs	Strong filthiness on The rear body	A cow hesitantly walks and saves one or more legs, has low and curved steps
2.	Larger hairless parts on hooks or thicker one or both legs	Clear contamination on the entire back legs	A cow walks unevenly or without pronounced slide movement lower head
3.	Larger areas without hair, 2-5cm	Detectable contamination	Cow walks, legs not quite parallel, head partially lowered
4.	Very small hairless parts, >2cm	Very small contaminations	A cow walks fluidly, straight back, head raised
5.	No hairless stains	Very Clean	A cow walks fluidly with Large-spaced steps

Luntz and Krogmeier (2019) concluded that the size of the herd affects skin and mobility problems, so larger herds have more problems with the skin and mobility. Finally, the amount of milk yield of cows, that is higher milk yield, doesn't have a negative effect on animal welfare criteria.

Conclusion

Based on this review it could be concluded that more attention is paid to animal welfare, through the education and training of people who deal with animals. Therefore, the morbidity of animals is reduced, reproductive efficiency is increased, the use of antibiotics is reduced, and the general health of animals that enter the food chain is improved. Also, the milk yield of dairy cows is increased which leads to higher profits. In that Way, animals are in excellent condition but also the farmers' satisfaction is increased.

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An analysis of breeding methods applied on the territory of the Nišava District

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Abstract

The main breeding objectives include increasing productivity, improving racial composition, and preventing the decrease in numbers when it comes to cattle breeding by genetically improving a breed, and honouring principles and regulations relating to animal welfare and environmental protection. The implementation of the main breeding programme for the Simmental breed which has been carried out for years on the territory of the Nišava District is in the function of improving cattle breeding by achieving breeding objectives and controlling production characteristics, as well as by breeding animals with desirable genetic potential from the standpoint of functional and physical development, with the preservation of the genetic variability of populations, reproductive and other traits of quality breeding animals. In the area of the Nišava District, there is a large number of heads that have not been assessed and they are not regarded as purebred regardless of their genetic potential. Production results that were monitored over a period of five years showed a continuous increase in the amount of milk by 14.23% compared to the initial year. Other parameters, proteins, milk fat, remained at the same level. After analysing the implementation of the breeding programme and selection measures in the period between 2015 and 2019 in quality breeding Simmental cattle that are under the supervision of the basic breeding organisation Veterinary Station Niš, Ltd. the following measures have been introduced: selection measures, linear assessment of first-calf heifers, and the control of milk yield in purebred cows. Bearing in mind that one of the important preconditions for a successful selection is the control of production traits of cattle heads, the research objective was to analysis implement zootechnical measures and possibilities increase the number of the herds under control in order to form the largest possible population of breeding cattle.

Key words: selection, breeding programme, selection roundup, linear assessment, milk yield control

Introduction

A breeding method implies a system of applied methods of the mating of selected cattle heads, i.e. it implies controlled reproduction of selected individual heads (Cunningham, E. P., 1981). By applying adequate breeding and selection methods, in line with the set goals, one can increase efficiency concerning milk and meat production, as well as concerning cattle breeding while preserving or improving their vitality, increasing efficiency, improving the quality of products, and preserving genetic diversity.

The cornerstone of breeding endeavours is a proper selection of animals that are to be the parents of future generations of offspring. Utilizing artificial insemination the largest genetic progress (as much as 85% among the Simmental breed population) is achieved by a proper selection of bulls´ and cows´ fathers. Even though at the population level one expects genetic progress of 15% by selecting bulls´ and cows´ mothers, these two selection processes are extremely significant for each breeder. The genetic progress of each breed depends equally on the breeding values of breeding males and females. In the area of the Nišava District, there is a large number of heads that have not been assessed and they are not considered purebred, regardless of their genetic potential (Khoda M. S., 2011). Bearing in mind that one of the important preconditions for a successful selection is the control of production traits of cattle heads, we resorted to increasing the number of the production stock to form as accurate a database as possible for the purebred stock.

The research goal was to implement zootechnical measures and increase the number of the production stock to form as accurate a database as possible for the purebred stock on the territory of the Nišava District. The selection of quality breeding female heads takes place in several steps. A young female breeding head whose parents have been introduced to the registry needs to be properly marked after birth, it cannot have any physical deficiencies, and it needs to be of an appropriate physical stature in accordance with the head age and breed standards. As an additional selection criterion one can resort to the data revealing the production and breeding value of parents. The classification of young female breeding heads into classes is done according to the rulebook which foresees minimum conditions for the classification of

young female breeding heads into classes (I, II, III), according to breeding directions.
Selection roundup – this measure is implemented to select the most suitable heads among all available breeding heads for the purebred stock. In cooperation with the regional breeding organization, we carry out a selection review in the area we cover by forming a selection commission using experiences from other countries of the world (Jabbar, M. A., 2010). The board comprises representatives from the basic and regional breeding organisations. When being assessed, cattle heads should be placed on a flat and not too hard a surface with good lighting. An animal should be well examined while at rest, as well as while in motion, and the board needs to provide mandatory information regarding the assessment date and the age of a cattle head (Pešić et al., 2020). Linear assessment is conducted after the first calving. It is done in relation to selected female heads, and once it has been finished the heads can be introduced to the herd book.

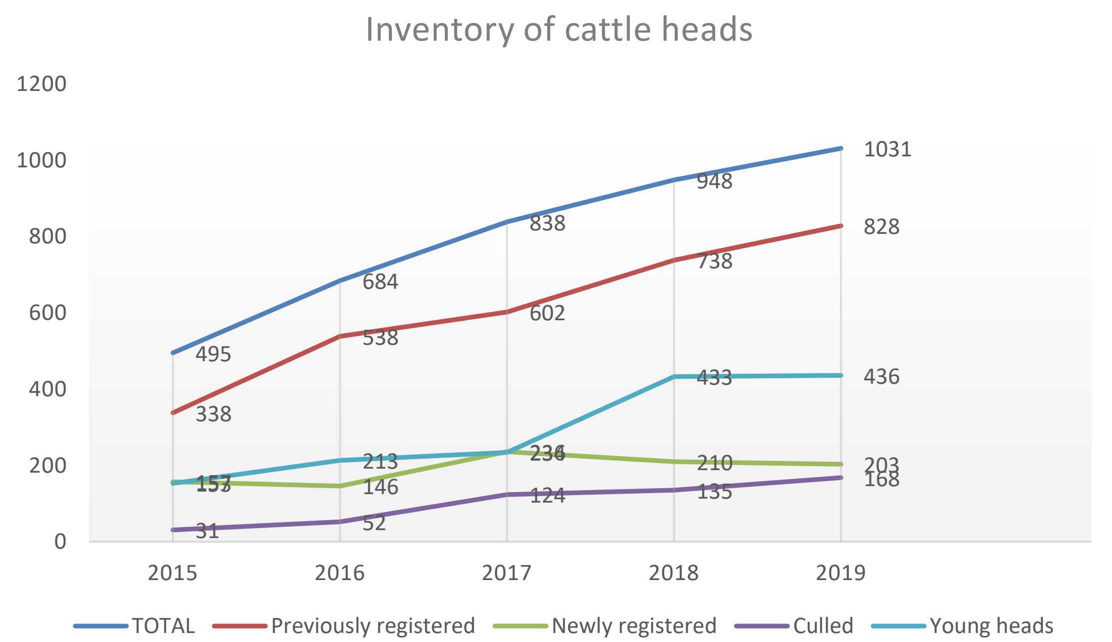
Materials and Methods

The basic measures for achieving the objectives foreseen by the breeding programme are as follows: breeding and production of quality breeding cattle of the Simmental breed, production of quality food, selection measures-selection programme, keeping pedigree records by controlling the milk yield, and determining linear measures. The material that was used during the analysis of breeding methods was taken from the archives of the Niš Veterinary Station, which refers to the Simmental breed of cattle. The analysis covered 3,996 heads of the Simmental breed of different categories.
The measures that were monitored were: selection reviews, linear evaluation of first-calf cows and control of milk yield of inbred cows. The monitoring period of the implemented breeding methods is from 2015 to 2019. Table 1 shows the scope of planned and implemented selection measures for the period between 2015 and 2019, whereby one can observe that the plan has almost been fully implemented. Graph 1 clearly shows an increase in the number of registered heads of the Simmental breed for the examined period between 2015 and 2019.

Table 1. The scope of planned and implemented measures in the period between 2015 and 2019

Measure	The scope of implemented measures														
	2015			**2016**			**2017**			**2018**			**2019**		
	A	**B**	**Ix**	**A**	**B**	**Ix**	**A**	**B**	**Ix**	**A**	**B**	**Ix**	**A**	**B**	**Ix**
Selection roundup	550	495	0.90	700	684	0.98	850	838	0.98	1000	948	0.95	1100	1031	0.93
Linear assessment of the first-calf heifers	150	112	0.74	200	137	0.70	200	149	0.75	250	145	0.58	250	187	0.74
Milk yield control in registered cows.	450	261	0.59	550	459	0.84	650	585	0.90	750	694	0.92	850	819	0.96

Source: Archive of the Veterinary Station Niš; Legend: A-planned, B-implemented; Ix-index



Graph 1. Total number of cattle heads in comparison to the number of registered cattle heads in the period between 2015 and 2019

The control of milk yield by applying the AT4 method is done following the main breeding programme (Petrović M., Pantelić V. 2015) . Milk yield control implies the collection of data on the amount of milk produced by cattle that are subject to breeding and selection work: milk control, control of milk fat and proteins, and control of the content of milk fat and proteins. According to the rules of the International Committee for Animal Recording (ICAR), the A4 method is considered to be the standard for implementing milk yield control, and it involves measuring the amount of milk in all cows on the control day in a period of four weeks on average. Taking into consideration economic, organisational, and technical possibilities in our country, this breeding programme foresees the use of the AT4 method for milk yield control. Milk yield control in all animals in the controlled herd is done according to the AT4 method. The first control needs to be done no earlier than 5 to 7 days after calving, no later than 15 to 51 days after calving, or in exceptional cases up to 80 days after calving (Stojanović M. et al., 2020).

The control is carried out alternately, one month in the morning and the next month in the evening. In exceptional cases, the control can be done twice in a row, in the morning and the evening, but not more than once a year (Lazić M. et al., 2018). The control is carried out in the allowed intervals, between two consecutive controls from 22 to 37 days. During lactation at least 11 controls are carried out. In the case of heads that run dry before the end of the standard lactation period of 305 days, for the lactation to be accepted, the minimum number of controls must be 9, and the lactation must not be shorter than 200 days.

Linear assessment – the assessment of the type, as well as of the physical development of breeding stock is carried out according to the established criteria, individually or in the course of selection roundups, by directly assessing the appearance and condition of a head. It is preferable to assess cows in the period of their first lactation from the 30th to the 150th day after calving, due to the activity and capacity of the udder in that period and the assumption that the genetic basis is safer for evaluation in that period, and that the influence of external factors is smaller. The linear assessment method System 97 intended for the Simmental breed constitutes the standard for representing the Simmental breed in Europe. The same system is applied in Germany, France, Italy, Croatia, Austria, Switzerland, Slovakia, Slovenia, the Czech Republic, and Hungary. The principle comprises linear assessment for each trait, from one extreme of the same trait to the other. Those 20 individual traits have been described and arranged into 4 basic categories: I – Framework; - the height of the withers, length of the pelvis, width of the pelvis, angle of the pelvis, depth of the body; II-Muscularity: muscularity; III-Foundation, the position of the hind legs, development of the hock joint, fetlock joints, height-appearance of the hooves; IV-Udder: front udder length, rear udder length, rear udder height, central ligament, udder depth, front udder teat position, teat position, teat length, teat thickness, udder cleanliness. Linear assessment includes the assessment of each predicted trait in its biological extremes, according to a scale ranging from 1 to 9 in the assessment card, whereby it should be emphasized that the highest score of 9 is not the most favourable for every trait.

For instance, the average score of 5 is the best for pelvic angle, the position of hind legs, and the length and thickness of teats. Certain characteristics of physical development are assessed by comparing them with possible biological extremes, regardless of the ideal model, and without prejudging the desirability of a particular characteristic. Although linear assessment does not describe the desirability of a trait, a score of 9 will represent either the most desirable score or the least desirable of the two possible extremes (Petrović M. and Pantelić V., 2015). When the positive biological extreme is undesirable, then the desirable property is located between the two extremes and it is most often evaluated with a score of 5, or some other score. A negative biological extreme is always undesirable. Each trait is therefore evaluated individually with a wide range of scores from 1 to 9, whereby the traits during the selection roundup are grouped by type, muscularity, foundation, and udder. In this way, one achieves greater accuracy, both in individual evaluations and in the overall external appearance.

The area in which the basic breeding organization Veterinary Station Niš Ltd. will carry out its breeding programme is that of the Nišava District, primarily the territory of the city of Niš with its associated municipalities, the municipality of Aleksinac and the municipality of Doljevac. On the territory of the city of Niš, there are 71 villages. About 3,500 heads of cattle are raised here, 90% of which are of the Simmental breed. On the territory of the municipality of Aleksinac, there are 72 villages. About 8,000 heads are raised there, 90% of which are of the Simmental breed. On the territory of the municipality of Doljevac, there are 16 villages. About 1,000 heads are raised there, 90% of which are of the Simmental breed.

Results and Discussion

After analyzing the implementation of the breeding program and selection measures from 2015 and 2019 in quality breeding heads of the Simmental breed under the control of the basic breeding organization Veterinary Station Niš Ltd. the following measures have been applied: In 2015, the following measures were carried out: Selection roundups of 495 Simmental heads, whether they be old or new in the registry; 338 previously registered heads and 157 newly registered heads. In 2015, there were 31 culled cows, while the number of cows for further reproduction in the breeding stock was 153. There were 112 linearly assessed first-calf heifers. Milk yield control was done for all purebred cows. In 2015 there were 261 completed lactations, 88 of which were first-calf heifers. The average duration of lactation was 323 days, the average production of milk during lactation per head was 4,261 kilograms of milk, and the average production of milk fat per head was 177,015 kilograms or 4.17%. The average protein production per head was 142,015 kilograms or 3.33%. Milk production in a standard lactation of 305 days was 4,019 kilograms with a fat content of 4.17%, and protein content of 3.33%.

Table 2. Achieved production results of embedded necks in 2015

Production results in 2015					
Lactation milk	Lactation milk	Lactation milk		Lactation milk	
dana	grlu	kg	%	kg	%
305	4,019	166,961	4.17	134,100	3.33
323	4,261	177,015	4.17	142,015	3.33

In 2016, the following measures were carried out: Selection roundups of 684 Simmental heads, whether they be old or new in the registry; 538 previously registered heads and 146 newly registered heads. In 2016, there were 52 culled cows, while the number of cows for further reproduction in the breeding stock was 213. There were 137 linearly assessed first-calf heifers. Milk yield control was done for all purebred cows. In 2016 there were 459 completed lactations, 174 of which were first-calf heifers. The average duration of lactation was 326 days, the average production of milk during lactation per head was 4,598 kilograms of milk, and the average production of milk fat per head was 193,216 kilograms or 4.20%. The average protein production per head was 149,805 kilograms or 3.26%. Milk production in a standard lactation of 305 days was 4,301 kilograms with a fat content of 4.20%, and protein content of 3.26%.

Table 3. Achieved production results of embedded necks in 2016

Production results in 2016					
Lactation milk	Lactation milk	Lactation milk		Lactation milk	
dana	grlu	kg	%	kg	%
305	4,301	182,448	4.20	141,176	3.26
326	4,598	193,216	4.20	149,508	3.26

In 2017, the following measures were carried out: Selection roundups of 838 Simmental heads, whether they be old or new in the registry; 602 previously registered heads and 236 newly registered heads. In 2017, there were 124 culled cows, while the number of cows for further reproduction in the breeding stock was 234. There were 149 linearly assessed first-calf heifers. Milk yield control was done for all purebred cows. In 2017 there were 585 completed lactations, 198 of which were first-calf heifers.

Table 4. Achieved production results of embedded necks in 2017

Production results in 2017					
Lactation milk	Lactation milk	Lactation milk		Lactation milk	
dana	grlu	kg	%	kg	%
305	4,573	189,091	4.15	144,732	3.18
329	4,916	203,971	4.15	156,121	3.18

The average duration of lactation was 329 days, the average production of milk during lactation per head was 4,916 kilograms of milk, and the average production of milk fat per head was 203,971 kilograms or 4.15%. The average protein production per head was 156,121 kilograms or 3.18%. %. Milk production in a standard lactation of 305 days was 4,573 kilograms with a fat content of 4.15%, and a protein content of 3.18%.

In 2018, the following measures were carried out: Selection roundups of 948 Simmental heads, whether they be old or new in the registry; 738 previously registered heads and 210 newly registered heads. In 2018, there were 135 culled cows, while the number of cows for further reproduction in the breeding stock was 433. There were 145 linearly assessed first-calf heifers. Milk yield control was done for all purebred cows. In 2018 the number of completed lactations was 694,177 of which were first-calf heifers. The average duration of lactation was 331 days, the average production of milk in lactation per head was 4,998 kilograms of milk, and the average production of milk fat per head was 206,649 kilograms or 4.15%. The average protein production per head was 158,649 kilograms or 3.17%. Milk production in a standard lactation of 305 days was 4,612 kilograms with a fat content of 4.13%, and a protein content of 3.17%.

Table 4. Achieved production results of embedded necks in 2018

Production results in 2018					
Lactation milk	Lactation milk	Lactation milk		Lactation milk	
dana	grlu	kg	%	kg	%
305	4,612	190,416	4.13	146,187	3.17
331	4,998	206,649	4.13	158,649	3.17

In 2019, the following measures were carried out: Selection roundups of 1031 Simmental heads, whether they be old or new in the registry; 828 previously registered heads and 203 newly registered heads. In 2019, there were 168 culled cows, while the number of cows for further reproduction in the breeding stock was 436. There were 187 linearly assessed first-calf heifers. Milk yield control was done for all purebred cows. In 2019, the number of completed lactations was 819, 204 of which were first-calf heifers. The average duration of lactation was 321 days, the average production of milk during lactation per head was 4,835 kilograms of milk, and the average production of milk fat per head was 198,839 kilograms or 4.11%. The average protein production per head was 152,712 kilograms or 3.16%. Milk production in a standard lactation of 305 days was 4.590 kilograms with a fat content of 4,11%, and protein content of 3,16%.

Table 5. Achieved production results of embedded necks in 2019

Production results in 2019					
Lactation milk	Production milk	Production milk fat		Production proteina	
days	$\overline{x}$	kg	%	kg	%
305	4,590	188,928	4.11	145,100	3.16
321	4,835	198,839	4.11	152,712	3.16

Conclusion

In comparison to previous years, in the period between 2015 and 2019, there was a significant increase in the number of registered Simmental heads included in the selection roundups of the basic breeding organization Veterinary Station Niš. This is a direct result of the Government's stimulus measures, as well as increased market interest. Despite the increase, the number is insufficient and below our country´s real potential for cattle production. Based on all the data presented here, it can be concluded that the set of measures for the implementation of the breeding program in the period from 2015 to 2019 has been implemented almost entirely according to the plan.

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Analiza sprovedenih odgajivačkih metoda na teritoriji nišavskog okruga

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Sažetak

Osnovni odgajivački ciljevi su povećanje produktivnosti, poboljšanje rasnog sastava i sprečavanje smanjenja brojnog stanja u govedarstvu putem genetičkog unapređenja rase, poštovanje principa i regulativa, vezanih za dobrobit životinja i zaštitu životne sredine. Sprovođenje glavnog odgajivačkog programa za simentalsku rasu, koji se godinama sprovodi na teritoriji nišavskog okruga, je u funkciji unapređenja govedarstva kroz postizanje odgajivačkih ciljeva i kontrolu proizvodnih osobina, gajenjem životinja poželjnog genetskog potencijala posmatran kroz funkcionalnu i telesnu razvijenost, uz očuvanje genetske varijabilnosti populacija, reproduktivnih i drugih osobina kvalitetnih priplodnih životinja. Na području nišavskog okruga postoji veliki broj grla koja nisu ocenjivana i ne nalaze se u matičnom zapatu bez obzira na njihov genetski potencijal. Analizirajući sprovođenje odgajivačkog programa i realizaciju selekcijskih mera od 2015 do 2019 godini kod kvalitetnih priplodnih grla simentalske rase koja se nalaze pod kontrolom osnovne odgajivačke organizacije ,,Veterinarska stanica Niš" d.o.o odrađen je sledeći obim mera: selekcijske mere, linearno ocenjivanje prvotelki i kontrola mlečnosti umatičenih krava. S obzirom na to da je jedan od bitnih preduslova za uspešno obavljanje selekcije kontrola proizvodnih osobina grla, cilj istraživanja je bio sprovođenje zootehničkih mera i omasovljavanje proizvodnog zapata kako bi se formiala što preciznija baza podataka za matični zapat.

Ključne riječi: selekcija, odgajivački program, smotre, linearne ocene, kontrola mlečnosti

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Comparative analysis of the production results of the Svrljig and Pirot sheep strains

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Abstract

The research presented in this paper aimed to determine which strain of Pramenka sheep achieved better production results under the same conditions of keeping, housing, care, and nutrition. In our country, the type of sheep with triple production capacities is the most widespread. Apart from the Pramenka breed originating from Svrljig, the Pirot Pramenka strain is represented in significant numbers as well, and it is mostly encountered in eastern and south-eastern Serbia, and found in the highest numbers in the highland region. The analysis of the comparative results of the two strains, the Svrljig and Pirot Pramenka strains, was carried out resorting to an experimental method on the Djordjević family farm in semi-extensive growing conditions. The heads of two strains were used as samples for the experiment: two-year-old Svrljig and Pirot sheep. The total number at the beginning of the experiment was: 243 heads of the Svrljig sheep strain, and 247 heads of the Pirot sheep strain. The results of the analysis showed that the Svrljig strain of Pramenka showed better results compared to the Pirot strain of Pramenka in terms of milk production, exceeding it by 1.08%, as well as in terms of the growth of lambs by 6.9%, and the amount of sheared wool by 2.3%.

Key words: sheep, Svrljig strain, Pirot strain, production, results.

Intraduction

Sheep breeding is a remarkably widespread branch of animal husbandry, but the importance of sheep breeding varies from one country to another. Sheep are widely represented and important in poor countries with extensive agriculture, but also in more economically developed countries (Gutić et al., 2006). Sheep consume low-quality bulky fodder and turn it into high-quality products such as meat, milk, wool, and fur. Milk is an important sheep product, and it is most often used in the form of cheese, while raw milk is rarely consumed. Meat is the most important sheep product, and the largest income in sheep production is generated through sheep or lamb meat. Nowadays, lamb meat is produced using different technologies, from pasture fattening together with grown sheep to intensive fattening in feedlots. What makes sheep unique and instantly recognisable is their wool. Sheep is characterised by wool production, whose economic importance is variable and differs from one country to another (Kukovic et al., 2013). In our country, the most widespread type of sheep is the one with triple production capacities. Apart from the Svrljig Pramenka strain which was most represented in eastern and south-eastern Serbia, and which can mostly be encountered in the highland region, the Pirot Pramenka strain was also represented in significant numbers. Both the Svrljig and Pirot Pramenka strains belong to the breed of triple production capacities, i.e. the breed of sheep for the production of meat, milk, and wool (Gorkhali et al., 2015).

The fact that the sheep, as a ruminant, can use coarse food of mediocre quality, and convert it into high-quality protein food for human consumption, makes the sheep a very useful domestic animal. By breeding sheep, one can obtain some high-quality, valuable products such as wool, milk, meat, fur, skin, horns, and manure. In the following presentation, we will get to know each strain individually (Pešić et al., 2020).

When considering the current situation in sheep breeding, one notices a significant reduction in numbers, weak racial composition, low production, unsatisfactory product quality, primitive cultivation, and lack of a development plan and programme. Almost entire sheep production is in the private sector, it is increasingly directed to basic subsistence, and the breeders themselves are unable to increase the production. There are economic incentives, but they are insufficient. In such circumstances, it is difficult to apply new scientific achievements on a larger scale and there is almost no monitoring of production and breeding heads (Momani Shaker et al., 2010). Therefore, it is necessary to organize producers, pool their resources and the resources of cooperatives and associations, and guarantee purchase at stimulating prices as well as product placement, which would undoubtedly increase the commodity of sheep production. The most represented domestic breed of sheep in Serbia is Pramenka, which makes up 80% of the total number of sheep. The second domestic breed in terms of number is the Cigaja breed, and it accounts for about 8.5% of cattle. In addition to these two breeds, in a relatively small number one can encounter about 0.5%, of the Lake-Solčava breed, and an insignificant number of domestic Merino sheep.

Pramenka is a term that denotes a primitive sheep, with weak physiological properties, which originated in poor conditions of nutrition and care, and which has well adapted to those conditions over the years. It is very resistant and strong. The Pramenka strain was once spread across the European continent. Intensive agricultural production resulted in suppressing the primitive Pramenka breed and substituting it with more productive breeds. Thus, it was preserved in a higher percentage only in the south of Europe, especially in the Balkan Peninsula (Gutić, 2006). In Serbia, Pramenka is cultivated in a very extensive way. During the summer period, it spends all its time on mountain pastures, which are usually scarce in valuable nutrients, due to the poor botanical composition of the pasture. In winter, they are kept in various facilities, awnings, and unsanitary barns, where they often starve. In autumn, many breeders of this particular breed of sheep, in search of food, leave mountain regions with their flocks to go to the plains several hundreds of kilometers away, because in these areas during the winter the sheep are usually not able to meet even the basic needs in nutrients.

In our country, there are several strains of Pramenka sheep, which differ from each other in terms of size, yield, quality, and wool colour. Common external features of all Pramenka strains are: long and narrow heads; sheep are hornless, less often horned, and rams are usually horned. All strains of Pramenka sheep are late-maturing animals. They reach sexual maturity at 16-18 months, and their growth ends at the age of 3-4 years. Pramenka is a sheep with combined production properties (meat-milk-wool). The production of Pramenka sheep and lamb meat is quite modest, depending on the strains and the level of nutrition, i.e. the degree of fattening of the animals. The live weight of sheep ranges between 25 kg and 55 kg, and rams between 35 kg and 80 kg. Meat yield, which ranges from 40% to 50%, depends on the same factors. The milk yield of Pramenka is relatively small, and it varies significantly between strains (from 40-100 liters and more during the six-months lactation period) and due to the influence of diet. The average weight of fleece is 1.4 kg. The yield of the wool ranges from 55% to 70%.

The fertility of the strain is relatively good. Some 100 to 110 lambs are obtained from 100 lambed sheep. The birth weight of these lambs is between 2 kg and 4 kg, which depends on the strain and the level of nutrition of the mothers. Lambs are hardy and mortality is low.

In our country, there is a large number of strains of Pramenka that originated in different climatic and nutritional conditions. They usually got their names according to the area of origin, a mountain, or some such. The most famous strains of Pramenka are Sjenica, Svrljig, Krivovir, Šar-Planina, Ovče polje, Kosovo, Metohija, Piva, and others. All Pramenka strains can be divided into two groups, namely the strains with soft curly wool and the strains with coarse wool.

The Svrljig Pramenka strain is an old population adapted to the conditions of the Svrljig region, which over time, due to improved conditions of nutrition and care, achieved visible progress in reproductive and production characteristics. The body weight of adult females is 50kg to 55 kg, and the weight of rams varies between 70kg and 90 kg. Fertility ranges from 110% to 115%. Lambs are born with a weight of 3 kg to 4 kg, achieving a daily gain of 180g to 220 g, so that at the age of 90 days they reach a weight of 20 kg to 23 kg. The body weight of a 12-month-old lamb is 40 kg to 45 kg. The milk yield is quite satisfactory and amounts to 80 to 100 liters in the period of lactation of 180 days. It is suitable for breeding in all areas and breeding systems, both extensive and intensive, for milk and lamb production. It is good as a maternal foundation in crossbreeding programs.

The Pirot Pramenka strain is the most widespread in the area of Pirot, after which it got its name. The height of its withers is about 55 cm. The body mass of adult sheep is 36 kg to 41 kg on average, and that of rams is 50 kg to 55 kg. The meat yield of adult heads is 45% to 47%, and that of lambs is about 50% to 52%, which depends on the degree of their fattening. Some 110 to 115 lambs are obtained from 100 sheep. The sheep are covered with white wool. Widely known Pirot carpets are made from the wool of the Pirot sheep grown in Pirot and its surroundings. In 6 months of lactation, this sheep produces 75 to 80 liters of milk, including the milk that the lamb sucks. It is a late-maturing breed. By resorting to the method of combined crossbreeding, with the application of rigorous selection, a much more productive genotype was created, the so-called Pirot sheep strain.

Material and Methods

The analysis of the comparative results of two strains of Pramenka, Svrljig and Pirot, was carried out using an experimental method on the Đorđević family farm, owned by Zoran Đorđević, an agricultural producer from the village of Bratiševac, near the municipality of Babušnica. .

The farm has a semi-extensive sheep farming system, so the herd spends most of the year on pastures, and stays in the barn during the winter. There are 2 facilities on the property: one is used for raising and fattening lambs, and the other for housing adult goats. Two strains were used as a test sample: two-year-old Svrliška and Pirot sheep. The total number at the beginning of the experiment was 243 head of Svrljiška sheep, and 247 head of Pirot sheep.

The aim of the research was to determine which strain of Pramenka sheep achieves better production results under the same conditions of keeping, housing, care and nutrition. Parameters that were used during the analysis are: milk yield of sheep and the amount of expressed milk, lambing index of sheep and growth of lambs. The amount of daily milk production was controlled by measuring in a milk meter, while the growth of the lambs was measured with a precise scale.

Results and Discussion

The research was conducted on the Djordjević family farm in the village of Bratiševac. We conducted a comparative analysis of two Pramenka strains, the Svrljig and Pirot strains. The research involved taking the production results of these two strains of Pramenka from the past three years (in the period from 2017 to 2019). Based on research results, we compiled a couple of tables in which we presented the production results of these two strains of Pramenka. In the tables, we entered data relating to the total number of heads, lambing index, number of lambs, amount of milk, growth of lambs, and weight of lambs at the final fattening. The data were entered for both Pramenka strains. Later, in other tables, we compared the obtained results of both strains and concluded which strain showed better results. When it comes to the production results of Svrljig Pramenka, the results of the test can be found in Table 1. The Svrljig Pramenka strain has adapted exceptionally well to the climatic conditions that prevail in the area of the village of Bratiševac, where the farm where we conducted the research is located. During this research, the results of sheep milk yield, lambing index, number of lambs, and growth of lambs were monitored. When we talk about the milk yield of this strain, the amount of milk depends on a number of factors, in addition to individuality, which is why it is different for different heads of the observed strain.

Table 1. Production results of the Svrljig Pramenka breed

	Sheep number	M	F	Lambing index	Lamb number	M	F	Milk yield	Lamb growth	M	F	Final fattening	M	F
Year	heads	heads	heads	%	heads	heads	heads	litres	gr.	gr.	gr.	kg.	kg	kg
2017	243	21	222	105	233	116	117	5750	330	385	275	19,10	22,05	16,15
2018	265	21	238	106	252	126	126	5810	325	375	275	19,05	21,05	17,05
2019	289	21	268	108	289	144	145	5865	345	405	285	19,30	22,20	16,40

Thus, the table shows the total amount of milk for the past three years, which ranges from 5600 l to 5900 l per lactation, that is, 22.29 litres of average production per head. The table also shows the lambing index, which ranges from 100% to 110%, with an average number of lambs of 1.05 lambs per head, as well as the total number of lambs. Also, the tables show the growth of the lambs and the weight of the lambs at the final fattening.

Table 2. Production results of the Pirot Pramenka strain

	Sheep number	M	F	Lambing index	Lamb number	M	F	Milk yield	Lamb growth	M	F	Final fattening	M	F
Year	heads	heads	heads	%	heads	heads	heads	litres	gr.	gr.	gr.	kg.	kg	kg
2017	247	23	224	108	242	121	121	5756	334	393	275	19,22	22,18	16,26
2018	271	23	240	102	244	122	122	5748	322	369	275	18,85	21,74	15,96
2019	294	23	271	105	284	142	142	5763	338	397	279	19,25	22,30	16,20

The presented results indicate that a modest way of feeding, primarily due to the composition of grasses from which the hay is made, as well as the quality of legumes, had an impact on the growth of lambs, as well as on the amount of milk per head. Deviation from the average production (theoretically) shown in the studies of a large number of authors just proves the results of our research.

Table 2 shows the production results of the Pirot Pramenka strain. Identical production indicators were also monitored for this strain of Pramenka for the same reason. The results are similar to the previous Pramenka strain. However, there are slight differences.

Table 3. Comparative results of the Svrljig and Pirot Pramenka strains (2017)

PRAMENKA										
2017	**Svrljig strain**					**Pirot strain**				
	n	**M**	**%**	**F**	**%**	**n**	**M**	**%**	**F**	**%**
Number	243	21	8,64	222	91,36	247	23	9,31	224	90,69
Body weight (>20kg)	116	22,05	18.97	-	-	121	22,18	18.18	-	-
Body weight (<20kg)	117	-	-	16,15	13.68	121	-	-	16,26	13.22
Fertility	233	-	-	117	50.21	242	-	-	121	50.00
Milk yield	5750	-	-	233	4.05	5756	-	-	224	3,89
Wool yield	225	3,40	1,57	2,27	1,01	227	1,76	0,77	1,40	0,62

When we talk about the milk yield, it is somewhat weaker compared to the previous strain, and the flow in the previous three years was in the range between 5700 l and 5800 l per lactation, which is 100 l to 200 l less, compared to the Svrljig Pramenka. However, it was found that meat production shows better results compared to the previous strain, which will be shown later in the research paper and tables. In the following tables, the obtained production results of both strains of Pramenka are compared. Production results that were monitored during the experimental period related to the amount of milk produced after the suckling of lambs, the weight gain achieved by the lambs, the number of lambs weaned, and the final weight of the lambs monitored. The period used to analyse the strands of Pirot and Svrljiška strains is three years, from 2017 to 2019. Comparative results are shown in the tables.

Table 4. Comparative results of the Pirot and Svrljig Pramenka strains (2018)

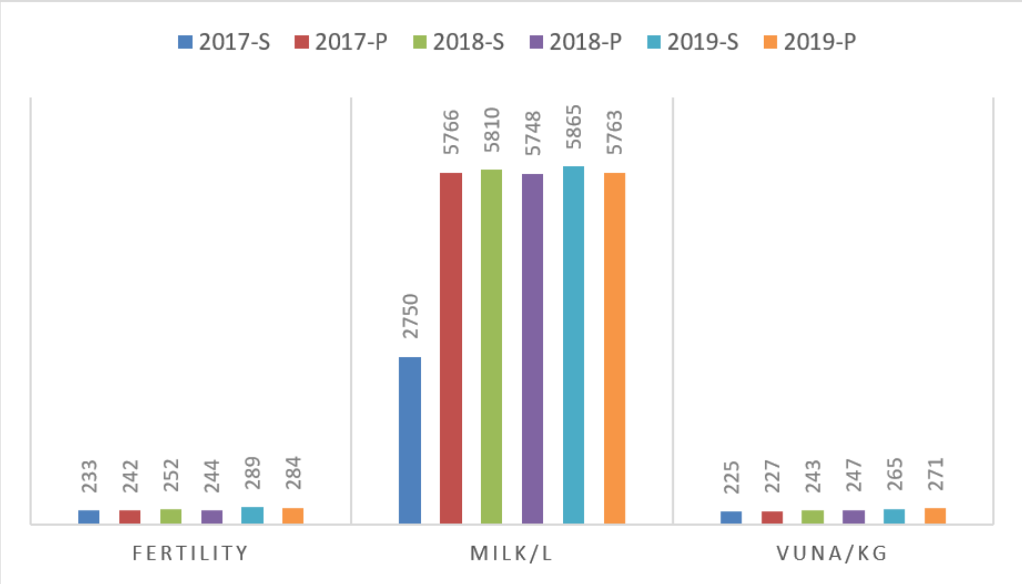
PRAMENKA										
2018	Svrljig strain					Pirot strain				
	n	M	n	M	n	M	n	M	n	M
Number	265	21	7,92	238	89,81	271	23	8,49	240	88,56
Body weight (>20kg)	126	21,05	16.67	-	-	122	21,74	17,21	-	-
Body weight (<20kg)	126	-	-	17,05	13.49	122	-	-	15,96	12.30
Fertility	252	-	-	126	50,00	244	-	-	122	50,00
Milk yield	5810	-	-	238	4.10	5748	-	-	240	4.18
Wool yield	243	3,39	1,40	2,25	0,93	247	1,72	0,70	1,38	0,56

By comparing the production results of two strains of Pramenka, the Pirot and Svrljg strains, it was determined that the Pirot strain showed better production results (Važić et al., 2004). The differences showed that the Pirot strain of Pramenka showed better results compared to the Svrljig strain of Pramenka in terms of milk production by 1.08% and growth of lambs by 6.9%.

Table 5. Comparative results of the Pirot and Svrljig Pramenka strains (2019)

PRAMENKA										
2019	Svrljig strain					Pirot strain				
	n	M	N	M	n	M	n	M	n	M
Number	289	21	7,27	268	92,73	294	23,00	7,82	271,00	92,18
Body weight (>20kg)	144	22,20	15.28	-	-	142	22,30	15.49	-	-
Body weight (<20kg)	145	-	-	16,40	11.03	142	-	-	16,20	11.27
Fertility	289	-	-	145	50.17	284	-	-	142	50.00
Milk yield	5865	-	-	268	4.57	5763	-	-	271	4.70
Wool yield	265	3,37	1,28	2,27	0,86	271	1,69	0,63	1,39	0,52

Larger quantities of milk produced in the flock of the Pirot strain by 1.56 liters per head compared to the Svrljig strain did not only affect the average milk production per ewe but also the fact that the suckling lambs had a larger amount of milk available, which contributed to greater food consumption, better feed conversion and higher growth of lambs. Naturally, all of the above caused a greater final mass in the lambs. Similar results were obtained by other scientists (Petrović et al., 2014). The obtained values are shown graphically in Graph 1.



Graph 1. Comparative results of the two strains of Pramenka; S – Svrljig; P – Pirot (2017-2019)

The results of these studies are also confirmed in the writings of other authors (Petrović et al. 2014). In that respect, it is necessary to improve the breeding conditions, and above all to improve the technology of sheep diet, considering that the variability of milk yield characteristics of 80% is the result of the influence of paragenetic factors, within which nutrition occupies a primary place (Pešić et al., 2020).

Conclusion

Pramenka (the Svrljig and Pirot strains) is of great importance for sheep breeding in eastern Serbia. Milk production is a very important feature of this breed, which is even nowadays, under the current breeding conditions, paid great attention to. Another important feature is meat production. In order to determine the milk yield and meat production of this strain, we conducted a systematic control of both the quantity and fat content of milk, as well as the quantity and quality of meat. Based on the conducted research, we can conclude the following: sheep production in the area of Babušnica, on the Stara Planina mountain, has a declining trend. The milk yield of sheep is within the limits of the breed capacity. However, with the improvement of fodder production and feeding technology, it can be much higher and more significant in the Pirot strain. The number of lambs obtained in the observed period was higher in the Pirot strain. The final weight of the lambs at lambing was higher in the Svrljig strain. In addition to facilitating the work on the farm, farm modernization would also affect the production results, which would be significantly higher. After consulting experts, we could improve the production results obtained on this farm. When it comes to the amount of wool obtained in the control period, we can conclude that the heads of the Svrljig strain produced a significantly higher amount of wool compared to the Pirot strain. Finally, by observing the comparative results, and bearing in mind everything mentioned in the work so far, we can conclude that the Svrljig strain has a slight advantage compared to the Pirot strain of Pramenka.

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Komparativna analiza rezultata proizvodnje pirotskog i svrljiškog soja ovaca

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Sažetak

Cilj istraživanja u ovom radu bio je da utvrdimo koji soj ovaca rase Pramenka postiže bolje proizvodne rezultate pri istim uslovima držanja, smeštaja, nege i ishrane. U našoj zemlji najviše je rasprostranjen tip ovaca trojnih proizvodnih sposobnosti. Osim svrljiške pramenke koja je bila najzastupljenija u istočnoj i jugoistočnoj Srbiji, koja je u najvećem broju zastupljena u brdsko-planinskom region i pirotski oplemenjeni soj pramenke je bio zastupljen u značajnom broju. Analiza uporednih rezultata dva soja Pramenke, Svrljiškog i Pirotskog sprovedena je eksperimentalnom metodom na farmi porodičnog poljoprivrednog gazdinstvo Đorđević u polu-ekstenzivnim uslovima gajenja. Kao material za eksperiment korišćena su grla dva soja: svrljiška ovca i pirotska ovac, starosti dve godine. Ukupan broj na početku eksperimenta je bio: 243 grla ovaca Svrljiškog soja i 247 grla ovaca Pirotskog soja. Rezultati analize su pokazali da je svrljiški soj pramenke pokazao bolje rezultate u odnosu na pirotski soj pramenke u pogledu proizvodnje mleka za 1,08%, prirasta jagnjadi za 6,9%, i količini nastrigane vune za 2,3%.

Ključne reči: ovce, svrljiški soj, pirotski soj, proizvdnja, rezultati

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Effect of terminal sire genotype on the carcass characteristics of the fattening pigs

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Abstract

The purpose of this study was to measure some of the carcass characteristics as meatiness, thickness of MLD and back fat in three different types of crossbred fatteners. Some of the characteristics of meat as color and pHi and pHu were also measured. All dams were the Yorkshire x Landrace and three different types of terminal sire (Duroc, Pietrain, Pietrain x Duroc). Immediately after farrowing, piglets were marked according to the crossbred which belonged. In total it was marked 188 (Yorkshire x Landrace x Duroc), 86 (Yorkshire x Landrace x Pietrain) and 443 (Yorkshire x Landrace x x Duroc) piglets. All piglets were reared on the same farm in the same condition. At the end of the fattening period the pigs were slaughtered at the local abattoir. In total 120 carcasses were measured. The results showed that the highest value of meatiness had the fatteners crossbred Yorkshire x Landrace x Pietrain on average 60.19%, while other two types of crossbred had the meatiness 59,06% and 58,61% (Yorkshire x Landrace x Duroc; Yorkshire x Landrace x Pietrain x Duroc) . The pHi, pHu and color of the meat derived from three types of crossbreed were very similar and there were no statistically significant differences.

Key words: terminal sire, carcass characteristics, fatteners

Introduction

The Republic of Serbia is a country with the estimated production of 3 million of pigs per year (Statistical Yearbook of the Republic of Serbia, 2021). The number of pigs has been in continuous fall in the last two decades. The data from 2006 showed almost 4 million produced pigs per year. It is a well-known fact that the biggest pig production in the Republic of Serbia was during the mid-1980s (Jeremić et al., 2018) when the export of pork meat was very developed. By the annual number of produced pigs, the Republic of Serbia is on the 11th place in Europe and ahead of some of the well-known traditional pig producing countries such as Hungary, Czech Republic, Bulgaria, Slovakia, Croatia (eurostat, 2019). Almost all ex-communist countries in Europe had the shrinking in the numbers and reduction in the pig production. The reduction of import taxes and opening of the markets in these countries in majority cases lost the competitiveness of their own production. The Republic of Croatia is an example of the former big pig producers in the ex-SFRJ Republic. In the last two decades the pig production has been reduced and from exporters this country became one of the big importers of pig meat and live pig for slaughter (Sviben, 2005). Moreover, even when the fatteners are produced it is done from imported piglets

The Republic of Serbia, especially Vojvodina, its North part, has almost the ideal condition for intensive pig production (Lierop et al., 2015). Currently the most prominent agricultural export is export of crops such as corn, instead of meat or meat products. During the good years Serbia has been one of top ten corn exporters in the word (USID, 2019).

The breeding program for pigs in AP Vojvodina is organized through the Main breeding organization for AP Vojvodina. The number of sows and boars under the control is in the continuous increase from 2013 (data from the https://www.stocarstvo.edu.rs/centar).

The lean meat content is an important characteristic of produced fatteners; the heritability for carcass quality is very high (Radović et al., 2020). However, the carcass classification of pigs is not adequately implemented into the payment of produced fatteners. The SEUROP standard of classification is used in EU countries. It was developed from the need to compare the price of pig's carcasses in EU member states. In the Republic of Serbia, the standard in partial use is from 1985, JUS standard (Petrović et al., 2009). There is not much data regarding the average lean meat content of the produced fatteners in the Republic of Serbia. One of the reasons is non-existence of permanent monitoring of lean meat content of fatteners. Another reason is outdated legislation which is currently in partial use. Comparing SEUROP standard and JUS standard meat content on the same carcasses was between 9.98% to 12.97% (Radović et al., 2020). The average lean meat content of carcasses (65,764) measured in the eight slaughterhouses in Vojvodina was 55.31% (Radović et al., 2021). In the research done in 2011 on 12.523 carcasses the average lean meat content was 52.29±2.04% (Dokmanović et al., 2013). However, there are huge differences in meatiness depending on the chosen method and device. Therefore, it is very hard to compare data and draw a conclusion on the average meatiness of slaughter fatteners. The aim of this study was to investigate the differences in quality of carcass and meat of the fatteners depending on the crossbreed, with the special point on the meatiness.

Material and Methods

Fatteners

The research was carried out on a farm in Vojvodina with a capacity of 1,600 sows. The farm has a complete production cycle from sows to fatteners and the farm does the selection of pure breeds to produce F1 gilts. The study included piglets-fatteners obtained from F1 generation sows and different terminal sire (Yorkshire x Landrace x Duroc; Yorkshire x Landrace x Pietrain; Yorkshire x Landrace x Pietrain x Duroc). For the purpose of this research piglets at the birth were marked depending on its crossbreed. In total it was marked 188 (Yorkshire x Landrace x Duroc), 86 (Yorkshire x Landrace x Pietrain) and 443 (Yorkshire x Landrace x Pietrain x Duroc). All fatteners were raised in the same premises and fed the same feed. The hogs' diet was based on the locally produced corn and soybean meals and was formulated to meet the animals' nutritional needs. The fattening of the hogs was divided in three phases: the starting, growing and finishing periods. After the end of the fattening period, when they were approximately six months old, they were taken in the local abattoir. In total 120 carcasses were measured for the purpose of this study.

Meatiness

The qualified operators performed the classification of pig carcasses. The operators had finished the training courses in Norway, in the agency for classification 'Nortura', and in Italy, in the organization of the Italian Ministry "Ministero delle Politic he Agricole Alimen- tary e Forestali" for which they received certificates. The lean meat content was measured on the slaughter line 45 minutes post mortem with the one-point puncture model. The measurement was carried out with an optoelectronic probe for the classification of pig carcasses CLASSPRO GmbH (Germany). The puncture was 7 cm from medial carcass line, between 3rd and 4th rib, from cardocranial point of view, perpendicularly to the carcass.

Ph

The initial and ultimate meat pH (pHi and pHu) of the musculus longissimus dorsi were measured using a pH meter (Testo 205, Testo AG, Lenzkirch, Germany). Meat pH were determined in triplicate, and the average values of three measurements were taken as a final result.

The color of meat

Instrumental pork color measurements were determined at 24 h postmortem after a standard 30 min blooming period by using NR110 Precision Colorimeter. Measurements were made by taking seven readings (technical replicates) on the surface of each meat sample. The average L*, a* and b* values of several measurements were taken as a final result.

Statistics

The basic descriptive statistical data analysis was done by using the software package TIBCO® Data Science Statistica™ 14 released in 2020 (TIBCO Software Inc., Palo Alto, California) The statistical significance of the differences between the mean values was determined using the t-test.

Results and Discussion

The carcass weights (average 85.15±1.25 kg), live weights (average 108.13±14.29) and cooled carcass weights (average 83.87±11.08) of all measured carcasses were very similar and there were no statistical differences (table 1). However, there were statistically significant differences on meatiness, MLD (M. longissimus dorsi, mm) and fat thickness on the back (mm). The highest value of meatiness had the fatteners crossbreed Yorkshire x Landrace x Pietrain, average 60.19%, while other two types of crossbred had the average 59.06% and 58.14%. The similar results were for the thickness of MLD, the highest value had the fatteners crossbreed Yorkshire x Landrace x Pietrain 62.52 mm and the smallest thickness of beck (12.91 mm).

Table 1. Meatiness and carcass characteristics of fatteners depending of terminal sire

crossbreed	YL*P	YL*D	YL*PD	∑
number	40	40	40	120
Carcass weight (kg)	85.76±2.59	85.41±2.15	84.48±1.16	85.15±1.,25
Live weight (kg)	108.91±13.96	108.48±15.18	107.87±14.17	108.13±14.29
Cooled carcass weight (kg)	84.47±10.83	84.14±11.77	83.66±11.01	83.87±11.08
Meatiness (%)	60.19±2.99[a]	59.06±2.78	58.14±3.43	58.61±3.30
MLD (mm)	62.52±6.80[a]	56.71±8.27	56.31±6.94	57.20±7.48
fat thickness on the back (mm)	12.91±3.36[a]	13.11±2.92	14.10±3.69	13.73±3.50

Legend: Y- Yorkshire, L- Landrace, P- Pietrain , D-Duroc

[a]- significantly differ at p=0.05

These results are in agreement with some previous research. The Kušec et al. (2004) found that the carcass of three way crossed fatteners with the Pietrain as a terminal sire to have lean meat on average 62.11%. Similarly, Edwards et al., 2003 found Pietrain progeny had a higher percentage of lean meat at slaughter than Duroc progeny.

Table 2. The color of meat, pHi and pHu

crossbreed	YL*P	YL*D	YL*PD
color			
L*	43.46	42.65	43.71
pH			
phi	6.11	6.04	6.09
pHu	5.73	5.77	5.81

Legend: Y- Yorkshire, L- Landrace, P- Pietrain , D-Duroc

In table 2 are present results for the color and pH of pigs meat. There were no statistically differences between the groups. The results indicated that the quality of meat is very similar between the crossbreed fatteners. This results are in agreement with some previous research (Jokanović et al., 2015).

From this results it seems that Pietrain as terminal sire give the fatteners with the highest lean meat content without undesirable meat characteristics. Meatiness and meat quality is one of the characteristics which are important in production of fatteners. For some of another characteristic important for production as survival rate in the condition on farms is better duroc as terminal sire (Pedersen et al. 2019). Moreover in research of Vidović et al. (2011) showed significantly less gain and needed longer period to reach a certain weight for the genotype of Pietren.

Conclusion

On the basis of the investigation of carcass and meat quality of crossbred fatteners originated from three different types of terminal sire it can be concluded that: The highest value of meatiness was observed in fatteners crossbred with Pietren as terminal sire, in average 60.19%. The thickness of MLD (mm) was also the highest in those fatteners and the lowest thickness of fat on the back. The quality of meat color, pHi and pHu were very much similar in all meat regardless of the crossbreed.

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Growth characteristics of calves given milk replacements with various protein and calorie levels

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Abstract

The goal of the study was to determine how various milk substitute formulations impacted the conversion, mass, and growth of calves. 116 male Holstein calves were divided into control and experimental groups during a period of 59 days. The experimental group's calves were fed a milk substitute that contained bacteria. There were no statistically significant differences between the groups at the end of the experiment, despite the control group's higher average body mass and average daily gain figures. The conversion of the milk substitute was lower in the experimental group. The cost of the total average growth of a male calf (EUR 51.8) of the Holstein-Friesian breed in the control group, is lower compared to the cost of a male calf (EUR 58.96) of the Holstein-Friesian breed in the experimental group.

Key words: calves; production characteristics; milk substitute

Introduction

Since milk substitutes began to be used, production technology has been constantly developing so that the milk substitutes we use today in feeding calves represent nutritionally valuable liquid feed.

(S.M. Grobler, 2008). In earlier research, different production results obtained from the use were recorded milk substitutes and milk. Some studies have shown better results in daily gain, body mass, and conversion of calves using milk compared to milk substitutes (Godden et al., 2005; Ivanković et al., 2013), while some studies resulted in better or uniform results

In production characteristics (Hill et al., 2008; El-Jack and Ahmed 2012). Drackley (2004) states in his work that lower daily gains, body weights, and feed conversion of calves were obtained in earlier research due to insufficient protein in milk substitutes. The same author states that energy also affects the mentioned production characteristics and that it is an important source of energy in the milk substitute, that is, it originates whether energy comes from lactose or fat. In some research, feeding calves a milk replacer with a higher protein level resulted in higher daily growth (Nonnecke et al., 2003; Cowles et al., 2006). More recently, diverse microbiological preparations such as yeasts, probiotics, and prebiotics are sometimes added to milk substitutes. Geiger et al. (2014) used a milk substitute with probiotics in their research and compared it with a milk substitute that contained a higher level of protein and energy and a milk substitute with a higher protein level that did not show better production characteristics. The aim of this work is to compare different types of milk replacers on daily gain, body weight, and conversion milk substitutes in calves.

Material and Methods

In total, 116 male Holstein calves were used in the study. Calves were brought from two farms. They were split into two groups: control (38 and 21 calves) and experimental (45 and 12 calves). The calves were 30 days old on average. The experimental group received one milk substitute that was continuously enriched with yeast microorganisms (*Saccharomyces cerevisiae*) during the whole experiment, while the control group received two milk substitutes (initial and final). The first, thirty-first, and sixty-ninth days of the trial saw each participant weighed individually. The calves were given a set amount of milk substitute each day, along with hay and a starting combination (18% crude protein), which they could eat at any time. Each animal's consumption of milk substitute was tracked on an individual basis using a trans. All data were processed with the STATISTICA program (StatSoft Inc. 2012).

Table 1. Content of nutrients and energy in milk replacements

Nutrients and energy	Control group		Experimental group
	0-30 days of age	30-60 days	0-60 days
	first milk replacement	second milk replacement	milk replacement with microorganisms
Crude protein (%)	22	22	21,5
Crude fat (%)	20	17	18,5
Crude fiber (%)	0	0,07	0,3
Metabolic energy MJ/kg	18,48	17,8	18,38

Results and Discussion

In calves from the first farm, the experimental group was significantly older at entry, with a mean age of 32 days (SD 6.3 days) (Student's t-test, p = 0.001). In the second farm (student's t-test, p 0.001) and Belje (student's t-test, p = 0.009), there was a significantly higher increase from the 30th to the 59th day in the calves of the experimental group. The price of a kilogram of calf growth (1.03 eur/kg) from the farms of the first farm in the control group, calculated on the basis of milk substitute consumption, is lower than the price of calf growth (1.09 eur/kg) in the experimental group. The cost of feeding per calf day (0.88 eur/FD) in the control group is lower than the cost of feeding per calf day (0.99 HRK/FD) in the experimental group. The cost of the total average gain of calves (51.8 euros) in the control group, calculated based on the consumption of milk replacers, is lower compared to the cost of calves (58.96 euros) in the experimental group. The cost price per kilogram of calf gain (0.89 eur/kg) from another farm in the control group, calculated on the basis of milk replacer consumption, is lower compared to the cost price of calf gain (0.93 eur/kg) in the experimental group. The cost of feeding per calf day (0.87 eur/FD) in the control group is lower than the cost of feeding per calf day (1.02 eur/FD) in the experimental group. The cost price of the total average gain of the calf (51.33 eur) in the control group, calculated on the basis of the consumption of milk replacer, is lower compared to the cost price of the calf (60.12 eur) in the experimental group. (Table 2).

Table 2. Average parameters in male Holstein-Friesian calves according to origin and type of milk replacement

Holstein-Friesian breed Male calves	Arithmetic mean (standard deviation)		Difference	95% confidence interval		P*
	Control	Experimental		From	to	
The First farm	n=38	n=45				
Age [days]	28 (5,5)	32 (6,3)	-4	-7,04	-1,92	**0,001**
Input mass [kg]	60,95 (6,8)	60,77 (7,1)	1,55	-3,18	3,00	0,953
Weight at the 2nd weighing (after 30 days) [kg]	81,24 (8,8)	80,96 (9,4)	2,02	-4,04	4,01	0,994
Gain until the 2nd weighing [kg].	20,3 (5,7)	20,1 (5,7)	0,15	-2,36	2,66	0,907
Gain until the second weighing (30 days) [kg/FD]	0,68 (0,2)	0,67 (0,2)	0,04	-0,08	0,09	0,954
Weight at the 3rd weigh-in (after 59 days) [kg]	111,11 (13,4)	114,77 (13,7)	3,02	-9,99	2,03	0,192
Gain from the 2nd to the 3rd [kg].	29,8 (6,6)	33,8 (6,4)	3,94	-6,79	-1,09	**0,007**

Holstein-Friesian breed Male calves	Arithmetic mean (standard deviation)		Difference	95% confidence interval		P*
	Control	Experimental		From	to	
Gain from the 2nd to the 3rd [kg/FD].	0,85 (0,2)	1,17 (0,2)	0,04	-0,41	-0,23	**<0,001**
The total amount of milk substitute consumed [kg]	33,0 (0,5)	33,1 (1,3)	-0,1	-0,51	0,36	0,729
Gain per feeding day [kg]	1,03 (0,2)	0,91 (0,2)	0,04	-0,14	0,01	0,093
Gain per head [kg]	50,16 (10,3)	53,96 (10,13)	3,797	-8,30	0,70	0,097
Cost per kg of growth based on consumption of MR, eur/kg	1,03	1,09				
FD cost price based on MR consumption, eur/kg	0,88	0,99				
The cost price of the total gain per calf based on the consumption of MR, eur	51,8	58,69				
The second farm	n=21	n=12				
Age [days]	35 (7,4)	33 (8,7)	2	-3,37	8,79	0,368
Input mass [kg]	57,95 (8,4)	57,59 (11,2)	3,55	-7,59	6,96	0,930
Weight at the 2nd weighing (after 30 days) [kg]	80,57 (10,9)	83,87 (17,1)	5,02	-12,25	8,31	0,697
Gain until the 2nd weighing [kg].	22,6 (5,6)	24,9 (10,3)	2,29	-8,00	3,41	0,417
Gain until the second weighing (30 days) [kg/FD]	0,75 (0,2)	0,88 (0,3)	0,10	-0,25	0,14	0,567
Weight at the 3rd weigh-in (after 59 days) [kg]	116,47 (15)	120,71 (27,6)	7,73	-20,06	11,59	0,588
Gain from the 2nd to the 3rd [kg].	34,6 (6,5)	36,9 (11,8)	2,26	-9,1	4,56	0,502
Gain from the 2nd to the 3rd [kg/FD].	0,98 (0,2)	1,27 (0,4)	0,10	-0,50	-0,08	**0,009**
The total amount of milk substitute consumed [kg]	32,6 (2,6)	33,9 (0,7)	-1,3	-2,91	0,39	0,130
Gain per feeding day [kg]	0,99 (0,2)	1,07 (0,3)	0,09	-0,25	0,12	0,473
Gain per head [kg]	57,84 (8,9)	61,76 (20,6)	3,92	-14,96	7,12	0,473
Cost per kg of growth based on consumption of MR, eur/kg	0,89	0,93				
FD cost price based on MR consumption, eur/kg	0,87	1,02				

Holstein-Friesian breed Male calves	Arithmetic mean (standard deviation)		Difference	95% confidence interval		P*
	Control	Experimental		From	to	
The cost price of the total gain per calf based on the consumption of MR, eur	51,33	60,12				

MR - milk replacement, FD - feeding days

Table 2 shows that in the first period of fattening from the first farm (first 30 days), the average daily gain was higher in the control group by 10 g/day (0.68:0.67 kg/day). In the second period, the average daily gain was higher in the experimental group by 140 g compared to the calves of the control group (1.17: 1.03 kg). The total daily gain of the experimental group was higher by 60 g compared to the calves of the control group (0.91: 0.85 kg/day).

In calves from another farm, the average daily gain in the first period was higher in the experimental group of calves by 130 g compared to the calves of the control group (0.83: 0.75 kg/day). In the second period, the average daily gain was higher in the experimental group of calves by 40 g compared to the calves of the control group (1.27: 1.23 kg/day). The total average daily gain is higher in the experimental group of calves by 80 g compared to the calves of the control group (0.99: 1.07 kg). The findings are consistent with those of Lee et al. (2008), who found no statistically significant differences in calf body mass. Geiger et al. (2014) obtained better results using a milk replacer enriched with microorganisms.

Conclusion

The calves of the experimental group fed with a milk substitute containing microorganisms had a higher final body weight than the calves of the control group, even though they had a lower level of crude protein in the mixture. The gain was lower in the experimental group in the first part of the experiment by 10 grams compared to the control group, while in the second farm, the gain was higher (24.9: 22.6 kg/FD) In the second part of the experiment, the gain of the calves in the experimental group was higher (300, 290 grams) than that of the calves in the control group, which is probably the result of lower energy content in the milk substitute of the control group. The calves in the experimental group used the nutrients from the meal better because a lower feed conversion per kg of gain was recorded. The obtained results clearly indicate that the use of microorganisms in milk substitutes improves the utilization of nutrients, which ultimately directly affects the reduction of greenhouse gases.

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Карактеристике раста телади којој су даване млијечне замјене с различитим разинама протеина и калорија

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Сажетак

Циљ студије био је утврдити како млијечне алтернативе различитих састава утјечу на претворбу, масу и раст телади. Тијеком 59 дана, 116 мушке Холштајн телади подијељено је у двије скупине: контролну скупину и покусну скупину. Иако су вриједности просјечне тјелесне масе и просјечног дневног прираста контролне скупине биле веће на крају експеримента, није било статистички значајних разлика између скупина. У покусној скупини дошло је до смањене конверзије млијечне замјене.

Кључне ријечи: мушка телад, производне карактеристике, млијечна замјеница

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The effects of different zeolit concentrations on hematological blood parameters in dairy cows

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Abstract

The aim of study is to examine the influence of zeolite addition to nutrition on the hematological parameters of blood of dairy cows. The research was carried out on 45 dairy cows of the domestic pied breed of the Simmental type. The animals were divided into 3 groups based on the amount of zeolite added to their feed mixtures. The first group of dairy cows (I-O group) had 4% Tufozel (the commercial name of zeolite) added to their feed mixtures during 15 months of treatment. The second experimental group of cows (II-O group) had 2% Tufozel added to their feed mixtures. The third group of dairy cows represented a control group (K-group) and did not receive Tufozel.

Hematological analysis of cow blood samples was performed at the beginning and at the end of the trial, by determination the hemoglobin content, and number leukocytes, erythrocytes and thrombocytes. The statistical analysis of the obtained data did not reveal any statistically significant differences between the groups.

Key words: cow, zeolite, hemoglobin, leukocytes, erythrocytes, thrombocytes

Introduction

Zeolite has been the subject of various types of research. It has a wide scope of application – it is used in the production of domestic animal feed mixtures, as well as a herbicide and pesticide carrier, for depositing radioactive waste, treating wastewater, cleansing waste gases, cleaning spilled oil, coal gasification, natural gas purification and exploitation, solar energy exploitation, oil production, fertilizer production, treatment of animal waste, water management, exploration of ore bodies, hydrometallurgy, construction, medicine, etc.

When it comes to livestock growing, various studies were conducted in relation to the production characteristics of dairy cows (Ilić et al., 2006 A), reproductive (Ilić et al., 2006 B) and health characteristics of domestic animals (Ilić et al 2007), as well as the environmental conditions in which the animals live.

It is already known that zeolite has certain positive effects on animal nutrition, but regardless of that, there is always room for new research

The aim of study was to examine the influence of zeolite addition to nutrition on the hematological parameters of blood of dairy cows”, to clarify the aim of study to the reader.

Material and Methods

The research included 45 dairy cows of the domestic pied breed. The cows were divided into 3 groups. There were 15 cows in each group and the experiment was carried out for 15 months. The groups were formed based on the amount of Tufozel mineral added to the feed mixtures. At the beginning of the experiment, the cows were in different stages of gestation. The experiment was carried out on family dairy farms in the village of Veliki Šiljegovac near Kruševac.

The first group of dairy cows (I-O group) had 4% Tufozel added to their feed mixture. The second experimental group of cows (II-O group) had 2% Tufozel added to their feed mixture. The third group of dairy cows was a control group (K-group) and did not receive Tufozel in their feed mixture. Tufozel is a zeolite brand manufactured by the "Nemetali" factory from Vranjska Banja. It is obtained by surface mining in Zlatokop near Vranje and is one of the highest quality zeolites in the world.

In this work, the following blood parameters were monitored: hemoglobin content, erythrocytes, leukocytes and thrombocytes.

At the beginning and at the end of the test, blood was drawn from the jugular vein of animals and analyzed. The obtained results indicate that the use of zeolite in the nutrition of cows has no harmful effects, taking into account the mentioned parameters. The blood was drawn from the jugular vein (V. Jugularis) of dairy cows at the beginning and at the end of the experiment. The collected blood samples were placed into sterilized and labeled tubes and preserved. The hematological analysis was carried out in the Kraljevo Health center. The following parameters were examined: hemoglobin content, erythrocytes, leukocytes and thrombocytes. The tests were performed usage of blood analyzer.

The results obtained during the research were processed using methods of descriptive statistics, and t-test for determination of statistical significance of differences, at levels of P<0.05 and P<0.01.

Results and Discussion

Hematological parameters of the blood were determined at the beginning and at the end of the experiment. From the hematological parameters of the blood of dairy cows of the domestic varicolored breed, the values of hemoglobin (the main protein in erythrocytes), leukocytes (white blood cells), erythrocytes (red blood cells) and thrombocytes (platelets) were determined.

The values of hematological parameters of blood in dairy cows given by Jovanović (1989) and Jazbec (1990) were taken as the reference values for the analyzed parameters.

Tables 1 and 2 show the concentrations of hemoglobin in the blood serum of cows at the beginning and at the end of the experiment.

Table 1. Hemoglobin concentration in the blood serum of cows at the beginning of the experiment (g/l)

Group	N	$\overline{x}$	$s_{\overline{x}}$	SD	CV(%)	Variation interval		F_{exp}
						Min	Max	
I-O (4%)	15	139.90	0.85	3.29	2.35	134.30	144.50	2.83^{NS}
II-O (2%)	15	142.23	1.43	5.55	3.90	134.30	153.00	
K	15	144.27	1.52	5.88	4.08	136.00	153.00	

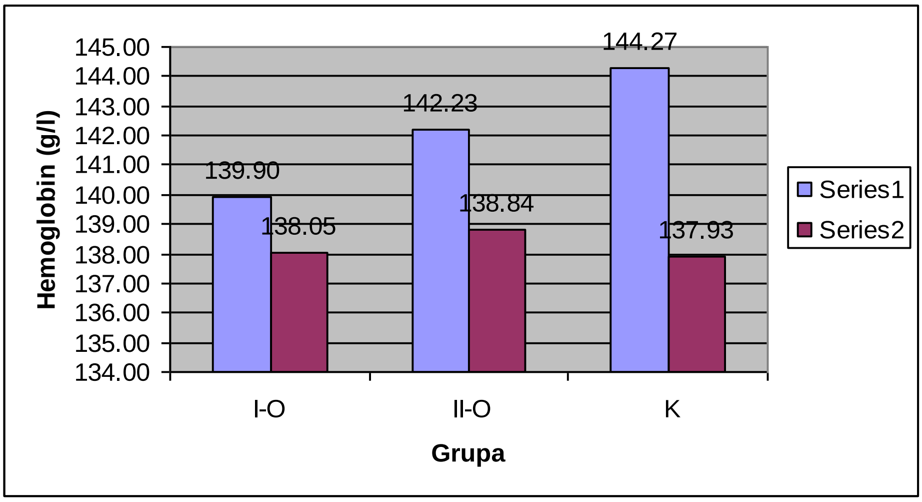
N.S. – P>0.05; * - P<0.05; ** - P<0.01; *** - P<0.001

Table 2. Hemoglobin concentration in the blood serum of cows at the end of the experiment (g/l)

Group	N	$\overline{x}$	$s_{\overline{x}}$	SD	CV(%)	Variation interval		F_{exp}
						Min	Max	
I-O (4%)	15	138.05	0.92	3.55	2.57	134.30	144.50	0.28^{NS}
II-O (2%)	15	138.84	0.74	2.87	2.06	134.30	143.00	
K	15	137.93	1.12	4.34	3.15	135.00	151.30	

N.S. – P>0.05; * - P<0.05; ** - P<0.01; *** - P<0.001
Reference value: 90-140 g/l of blood

Graph 1 shows the ratio values of hemoglobin concentrations in the blood serum in different cow groups at the beginning and at the end of the experiment.



Graph 1. Ratio values of hemoglobin concentrations in the blood serum in different cow groups at the beginning and at the end of the experiment

Series 1 - Haemoglobin concentrations in the blood serum of the cows at the beginning of the experiment

Series 2 - Haemoglobin concentrations in the blood serum of the cows at the end of the experiment

At the beginning of the experiment, the concentration of hemoglobin of the I-O group cows was at the upper physiological limit, while those values were slightly higher in the II-O and K-groups. At the end of the experiment, the hemoglobin concentration was within the reference values. Consolidation and equalization of the blood serum hemoglobin occurred between the groups at the end of the experiment, in comparison to the values measured at the beginning of the experiment. The statistical analysis determined that there were no significant differences between the observed groups at the beginning of the experiment, as well as at the end of the experiment. The obtained values were slightly higher than the values stated by Nešić (2000), and in terms of significant differences, the results were the same. There were no statistically significant differences.

Tables 3 and 4 show the concentrations of leukocytes in the blood serum of cows at the beginning and at the end of the experiment.

Table 3. Leukocytes number in the blood serum of the cows at the beginning of the experiment (in 1 l)

Group	N	$\overline{X}$	$s_{\bar{x}}$	SD	CV(%)	Variation interval		F_{exp}
						Min	Max	
I-O (4%)	15	6.28	0.20	0.77	12.26	5.30	7.80	1.35^{NS}
II-O (2%)	15	6.78	0.30	1.15	16.98	4.90	8.70	
K	15	6.82	0.26	1.02	14.90	5.20	8.40	

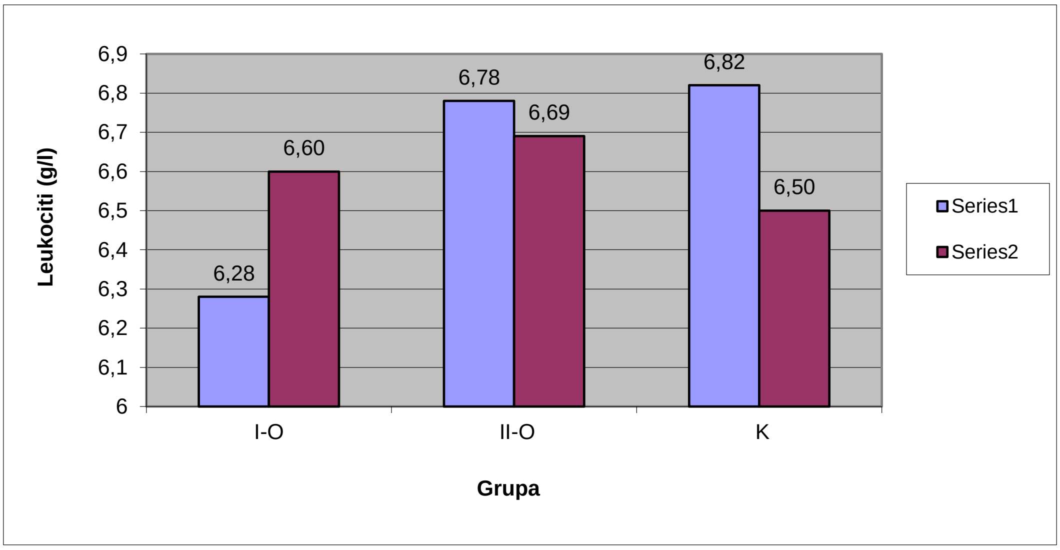
N.S. – P>0.05; * - P<0.05; ** - P<0.01; *** - P<0.001

Table 4. Leukocytes number in the blood serum of the cows at the end of the experiment (in 1 l)

Group	N	$\overline{x}$	$s_{\bar{x}}$	SD	CV(%)	Variation interval		F_{exp}
						Min	Max	
I-O (4%)	15	6.60	0.24	0.93	14.14	5.20	8.40	0.13^{NS}
II-O (2%)	15	6.69	0.27	1.03	15.41	4.90	8.40	
K	15	6.50	0.25	0.95	14.62	5.30	8.20	

N.S. – P>0.05; * - P<0.05; ** - P<0.01; *** - P<0.001
Reference value: 5.0-10.0 x 10^9

Graph 2 shows the ratio of leukocytes concentrations in the blood serum at the beginning and at the end of the experiment.



Graph 2. Leukocytes concentration in the blood serum of cows

Series 1 - Leukocytes concentrations in the blood serum of the cows at the beginning of the experiment

Series 2 - Leukocytes concentrations in the blood serum of the cows at the end of the experiment

The concentration of leukocytes in all groups of cows was within the reference values at the beginning of the experiment, as well as at the end of the experiment.

Statistical analysis revealed that there were no significant differences between the observed groups both at the beginning and at the end of the experiment.

Tables 5 and 6 show the concentrations of erythrocytes in the blood serum of cows at the beginning and at the end of the experiment.

Table 5. Concentration of erythrocytes in the blood serum of the cows at the beginning of the experiment (in 1 l)

Group	N	$\overline{x}$	$s_{\bar{x}}$	SD	CV(%)	Variation interval		F_{exp}
						Min	Max	
I-O (4%)	15	6.55	0.12	0.45	6.94	5.90	7.40	2.03^{NS}
II-O (2%)	15	6.32	0.26	1.01	16.02	5.18	8.61	
K	15	6.99	0.30	1.45	16.43	5.39	8.82	

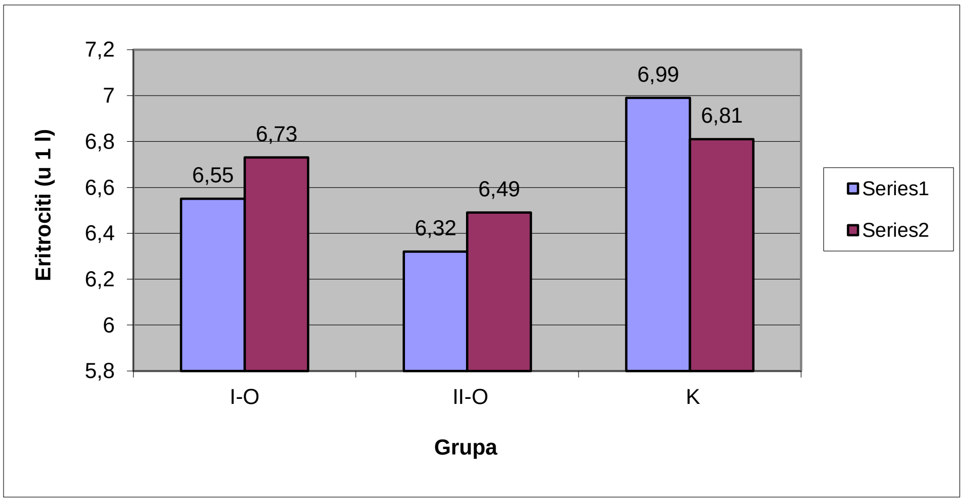
N.S. – P>0.05; * - P<0.05; ** - P<0.01; *** - P<0.001

Table 6. Concentration of erythrocytes in the blood serum of the cows at the end of the experiment (in 1 l)

Group	N	$\overline{x}$	$s_{\bar{x}}$	SD	CV(%)	Variation interval		F_{exp}
						Min	Max	
I-O (4%)	15	6.73	0.16	0.60	8.99	5.49	7.86	0.5^{NS}
II-O (2%)	15	6.49	0.25	0.95	14.68	5.25	8.61	
K	15	6.81	0.29	1.12	16.49	5.39	8.82	

N.S. – P>0.05; * - P<0.05; ** - P<0.01; *** - P<0.001
Reference value: 5.0-8.5 x 10^{12}

Graph 3 shows the ratio of erythrocytes concentrations in the blood serum at the beginning and at the end of the experiment.



Graph 3. Erythrocytes concentration in the blood serum of cows

Series 1 - Erythrocytes concentrations in the blood serum of the cows at the beginning of the experiment

Series 2 - Erythrocytes concentrations in the blood serum of the cows at the end of the experiment

The concentration of erythrocytes, in all groups of cows was within the reference values. There were no statistically significant differences between the groups at the beginning of the experiment, as well as at the end of the research.

Tables 7 and 8 show the concentrations of thrombocytes in the blood serum of the cows at the beginning and at the end of the experiment.

Table 7. Concentration of thrombocytes in the blood serum of the cows at the beginning of the experiment (in 1 l)

Group	N	$\overline{x}$	$s_{\overline{x}}$	SD	CV(%)	Variation interval		F_{exp}
						Min	Max	
I-O (4%)	15	313.00	12.78	49.48	15.81	215.00	369.00	1.70^{NS}
II-O (2%)	15	320.40	18.08	70.01	21.85	198.00	428.00	
K	15	283.60	12.77	49.47	17.44	175.00	354.00	

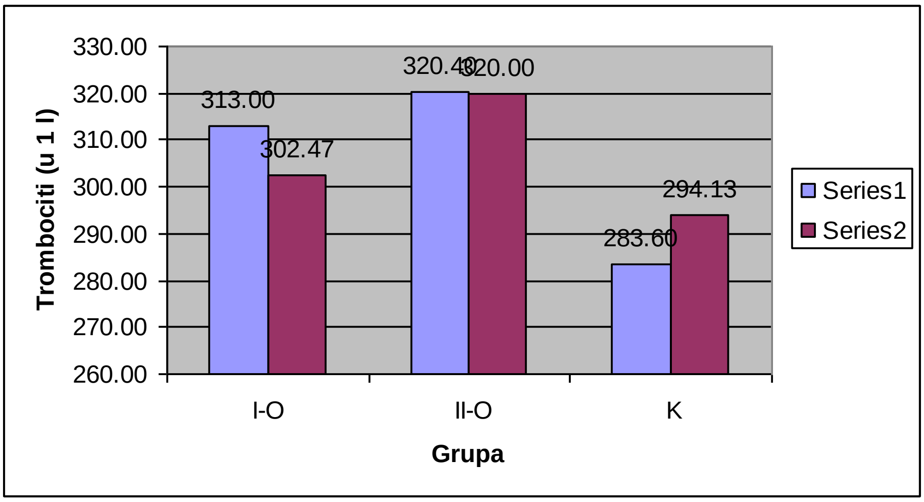
N.S. – P>0.05; * - P<0.05; ** - P<0.01; *** - P<0.001

Table 8. Concentration of thrombocytes in the blood serum of the cows at the end of the experiment (in 1 l)

Group	N	$\overline{x}$	$s_{\overline{x}}$	SD	CV(%)	Variation interval		F_{exp}
						Min	Max	
I-O (4%)	15	302.47	11.62	44.96	14.87	215.00	396.00	0.97^{NS}
II-O (2%)	15	320.00	14.26	55.18	17.22	205.00	320.40	
K	15	294.13	14.85	57.45	19.53	175.00	395.00	

N.S. – P>0.05; * - P<0.05; ** - P<0.01; *** - P<0.001
Reference value: 260-700 x 10^9

Graph 4 shows the ratio of thrombocytes concentrations in the blood serum at the beginning and at the end of the experiment.



Graph 4. Thrombocytes concentration in the blood serum of cows

Series 1 - Thrombocytes concentrations in the blood serum of the cows at the beginning of the experiment

Series 2 - Thrombocytes concentrations in the blood serum of the cows at the end of the experiment

Statistical analysis has shown that there were no significant differences between the observed groups. Statistical significance was manifested neither at the beginning nor at the end of the experiment.

The results obtained in these studies were within the limits of physiological values for the analyzed parameters according to the reference values stated by Jazbec (1990). Jovanović (1989) stated the number of erythrocytes in 1 l of blood (1000 ml) to be 7 (5-9) x 1012, the number of leukocytes in 1 l of blood to be 8 (6-10) x 109, the number of platelets in 1 l of blood to be 260-700 x 109 and 120 g (90-140) of hemoglobin per 1 l of blood. According to Stojić (1996), the number of erythrocytes in 1 l of blood in cattle is 6-8 x 1012, the number of leukocytes in 1 l of blood is 6-10 x 109, of which 25-35% are neutrophilic, 2-5% eosinophilic, 1% basophils, 60-65%, 60-65% lymphocytes and 5% monocytes. Their percentage ratio is called the leukocyte formula. The results presented in this paper are in accordance with the results obtained by Nešić (2000).

Conclusion

Based on the research of the influence of different concentrations of Tufozel-type zeolite on the hematological parameters of the blood of dairy cows and the results obtained during the research it can be concluded that hematological blood parameters determined at the beginning and at the end of the experiment indicate that zeolite added through feed mixtures had no effect on them and that these hematological blood parameters were within the reference values and that there were no statistically significant differences between the groups.

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Positive interactions between horse and rider

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Abstract

The aim of the work was to present a way of developing positive interaction between horses and riders in training. Interactions between horse and rider that lead to a positive relationship are realized through a series of human actions towards the horse and vice versa. The interaction between horse and rider is influenced by many factors; rider's seat, training method, appropriate equipment and knowledge of the basic characteristics of horse ethology. The connection between a man and a horse is the embodiment of the greatest connection between a man and an animal, while the ultimate goal of riding is to achieve union with the horse, that is, some kind of fluidity. With proper work and training, horses show a desire and willingness to communicate with people, while coercion in working with a horse is the worst for both partners. The rider around the horse should have the qualities of a calm and reliable leader, while the horse should have confidence in its rider. Constant proper work and time dedicated to the horse will lead to a high-quality and successful creation and maintenance of a positive relationship between horse and rider.

Key words: horse, training, positive interaction

Introduction

Horse riding is a popular sport and form of recreation for people all over the world. Throughout history, horses have played various roles. Many wars were won on their backs, while today they are used for recreation and people keep them primarily out of love. The most popular activity with horses is horseback riding. Horse riding can be recreational or disciplinary. In riding, the rider controls the horse's movements in several ways. I apply signals through my weight, i.e. the seat, pelvis, arms and legs in order to encourage the horse to move in the given direction, speed and manner. A well-ridden horse reacts to very light signals given by the rider, and the rider is able to sit independently of the horse. In order for the relationship between individuals of two different species to be good, it is suggested to achieve a positive relationship with the horse. The relationship between humans and horses is based on several interactions between them. The outcome of these interactions will lead to a positive or negative relationship. Such a relationship between horse and rider is of increasing interest among riders, but also a relatively new field of science (Hausberger et al., 2008, Andersen, 2015). The relationship between horse and rider is interesting and still largely unexplored. In any case, it always needs to be improved. It is a mistake to see riding as a horse's job because riding is a privilege. Interactions between horses and humans take place in many ways, through feeding, grooming, treatment and riding. The outcomes of these interactions can be positive or negative, depending on a number of factors. The aim of the work is to clarify the interaction between horse and rider and how the relationship between them is realized.

The relationship between horse and rider

The interaction between horse and rider falls under the study of anthrozoology, which investigates communication between animals and humans. Although a relatively new field of science, horse-rider interactions have captured the interest of riders and scientists (Andersen, 2015). Horse riding is quite a dangerous sport and in terms of frequency of injuries, it competes with football, motorcycling, rugby and skiing. In order to minimize the number of injuries, it is necessary to develop a positive relationship and interaction with the horse. It can be achieved by choosing a certain training method and implementing it. The interaction between horse and rider is influenced by many factors; the rider's seat, training method, appropriate equipment and knowledge of the basic characteristics of horse behaviour. Through his seat, pelvis, arms and legs, the rider signals the horse at what speed and in which direction he wants it to move (Andersen, 2015). Before riding, it is necessary to check the correctness of the rider's bridle and saddle, as these are the main points of contact between the horse and the rider. The saddle must fit the back of the horse as well as the rider. That is why it is important to choose a horse that matches the rider's size, and weight and is within the 20% rule. The 20% rule refers to the weight and requires that the rider can weigh a maximum of 20% of the horse's weight (National Equine Forum, 2022). An ill-fitting saddle can cause pain and will lead to a negative interaction between rider and horse. The relationship between horse and rider is a relationship of mutual dependence, cooperation and mutual becoming (Maurstad et al, 2013). The relationship between man and horse is considered an equal partnership. It must be taken into account that riding is an activity in which two individuals of different species participate and that their communication is still a big unknown. Mastering riding is a long process that requires constant improvement. Discovering new knowledge in working with horses also brings new methods in training. An experienced rider, as well as an inexperienced one, is expected to constantly work with a trainer and learn new things in order to improve their skills, communication with the horse and performance. During training, it can happen that the trainer asks the rider to get off the horse because the rider fails to perform a certain exercise. This is often the result of poor communication between the rider and his horse. In that case, the horse perceives the trainer as a person with greater authority and will perform the task more obediently and efficiently (Lesimple, et al. 2012). The connection between man and horse is the embodiment of the greatest connection between man and an animal (Fenner et al. 2020). Communication between horse and rider takes place through the bodies of both partners, requiring sensitivity, emotion and kinetic intelligence (Maurstad et al., 2013). The ultimate goal of riding is to achieve union with the horse, i.e. a kind of fluidity with its body. To achieve this goal, it is necessary to have a feeling for the animal you are working with. Feeling for the horse is something that every rider strives for, and it signifies a form of non-verbal communication between the two species, relying on body language, mutual understanding and cooperation (Fenner et al. 2020). The horse should accept the man as part of the herd. Horses, tame or wild, tend to form social bonds with each other in a herd. They achieve social connection in several ways; by sending visual messages (e.g. throwing the ear back), neighing and growling, smelling excrement and urine and grooming, and body position. Horses that have not been trained by humans do not know how to recognize the happy or angry or raised tone of voice and do not understand our words, which in other words would mean that people effectively use only a very small part of the horse's "language" when communicating with them (Turner, 2022). This is quite normal considering that horses and men are different species. When working with a horse, the rider should be authoritative, and reliable, and the horse will trust not out of fear but out of the safety of the leader. The leader of a herd of horses in the wild is a stallion or an experienced mare whose main role is to reproduce and protect the herd. Hierarchically below him are one or two mares whose role is to find food and water. The importance of the alpha stallion or alpha mare in the herd is equal, and they have acquired better physical fitness than other individuals through experience (Cathy, 2022). A horse will follow the one with whom it feels safe and cared for. The horse should look at its rider so that he is a leader who will not harm him. Gaining mutual trust is a long-term process between man and horse, and it starts from foaling. Socialization is the primary goal in raising foals, and it is necessary to spend as much quality time with them as possible. In order to create a stable and courageous horse, it is necessary to work with him in the early stages of his life. It's never too early to start teaching your foal some unusual objects or things that make different sounds. Walking young horses in hand is also laying the foundation for riding. The horse gets to know its surroundings and separates itself from the only things it has ever known, which are the stable, the familiar yard and the drain. In the aforementioned walks, they will encounter many unknown and frightening things that the owner must familiarize the young horse with so that he will approach the object first, and then let the horse sniff and explore (Socializing foals, 2022). In these situations, patience and persistence is the most important item. With this combination, the development of positive interaction with the horse will be achieved. After mastering the aforementioned basics of socialization and developing a positive relationship with the horse, you can start riding. There are several disciplines and types of riding, but this thesis is based on newer and more modern methods, with the mention of classic ones.

Horse training methods

The first written record of horse training is mentioned in 1350 BC by Kikkuli, who is still known as the "chief horse trainer" in the Hittite Empire. It can be said that the horse is the animal on whose back the entire history of mankind is written. Horses had various roles in the past that are practised less today. For people, they were a source of food, a means of transportation and a necessary tool in war. Today, keeping a horse is considered a luxury and with that, the very purpose of keeping a horse has changed. For hundreds of years, horse training methods did not change, everything was based on traditional horse training. In 1980, newer methods of horse training appeared and became more and more popular. Examples of where traditional training methods differ from modern ones are numerous.

In accordance with the ethical guidelines set by the International Society for Applied Ethology (ISAE, 2022), work with horses focuses on ethical horse training, improved horse behaviour and welfare, improved understanding of human-horse interaction, facilitating scientific education about riding and development of horse research methods and techniques. Working with a horse should be guided by the safety of man and horse, the natural needs of the horse (food, physical activity, contact with other horses), the mental abilities and perception of the horse, maintaining a positive state through the work, avoiding pain, discomfort and stress. pressure on the horse (related to the possibility of escape). While working with the horse, understand that the behaviour becomes more or less likely depending on the consequences, strict training should release the pressure as soon as the desired behaviour appears, avoid delaying the reward, use a combined reward and avoid punishment. Through work with the horse, desensitization should be achieved so that an unpleasant situation can lead to an urge. Modelling training into smaller segments to achieve dynamics. Each step (segment) to the desired action ends with a reward. Dynamics can be achieved by changing only one training segment (trainer, place, signal). Plan the training so that it is understandable and easy for the horse. Through training, the horse needs to develop self-confidence. To achieve interaction with the horse, the signal should be clear and understandable to the horse. Check if the horse can distinguish one signal from another. For successful signal communication, ensure that each signal has only one meaning, match the signal with the biomechanics of the horse, and avoid using multiple signals at the same time (ISAE, 2022).

Horse training in the narrower sense refers to accustom the horse to equipment, lunging and mounting and riding. The basic equipment required for riding consists of a bridle with bit and stirrup, saddle pad, gel, saddle, belt and protector. Lunging a horse can be done in several ways, and the equipment for this is quite diverse. To lunge a horse without a jaw, the equipment includes a halter for lunging, lunge, guards and a long whip. A lanyard and harness can be used, and instead of a halter, a bridle with a bridle (HKS, 2015).

Lunging is the basis of training a young horse, and the purpose of lunging is numerous. Some of the reasons why it is necessary to lunge horses are:

- To improve rhythm and balance in the walk, trotting and canter
- To tire a spirited horse before riding
- For examining lameness in horses (HKS, 2015).

After the horse is familiar with the equipment used for riding, it is necessary to teach him to lunge. The lonža is a strap approximately eight meters long, it is attached to the ring of the jaw, and it is held by the trainer (HKS, 2015). During lunging, it is essential to use voice commands. For example, when the horse is asked to walk calmly and "walk" is said slowly when the trainer wants the horse to trot excitedly, he will quickly say "trot" so that the horse will feel in his voice that he is being asked to move at a faster pace. A young horse should not lunge for too long due to the pressure on the joints that occurs when moving in a circle. When the horse adopts certain commands, it should start with saddling and lunging under the saddle (Mitrović, 2003). A horse that has been trained on a lunge harness accepts the saddle mostly without problems.

When the horse has mastered voice commands and lunging with the saddle, it is ready for its first ride. You should be careful during the mounting procedure (Gregić et al, 2017). For riding a horse, it is safer for the horse to be attached to the longe. A minimum of two people are required when climbing a horse for the first time. Before actually sitting on the horse, it is suggested to lean your stomach on the horse's back in order to feel the rider's weight. Thus, there is a possibility to get off the horse quickly if he gets scared. The first rides are done on small fenced areas and start with a light step of the horse. After a few days, a light trot is gradually introduced, followed by a gallop. After the horse gets used to the rider, after a few days, if it is calm during riding, the halter can be removed and riding continues in a circle without the halter. When we assess that the horse is prepared, it can be moved to a larger open riding area (Gregić et al., 2022).

Horse management is done in several ways. Most people think that the rider's bridle with the bit is the priority in handling the horse, but this is not necessarily true. Equally important is the rider's leg, i.e. calf and seat, as well as voice. The rider's legs control forward motion, turning and balance. The inside leg of the rider is used to encourage the horse to move forward, to quicken the stride or to emphasize the turn. The rider's outside leg is used to control the horse's hindquarters. If the rider's inner and outer leg commands are used at the same time, the horse's balance is achieved. The reins attached to the bit are used to maintain contact with the horse, and by gently pulling them, we let the horse know when we want to turn right, left or stop (Gregić et al., 2022). The jaw itself should not be considered a steering wheel or a brake on a horse. To reduce the pressure on the horse's mouth, it is necessary to learn to follow the commands of our body. For example, if the rider wants to go from a trot to a gallop, he will lean his whole body forward. When turning to the left, the rider slightly shifts the entire body weight to the left side of the saddle. By perfecting all the mentioned methods of horse management, the rider will create a perfectly ridden horse.

The rider's seat does not refer exclusively to the part of the body that sits in the saddle, the seat is considered everything from the lower part of the abdomen and waist to the knees. The balanced seat allows for a good rider's stance, i.e. holding on to the horse, and loose and supple legs ready to change the speed at any time. It is also useful when staying on a restless horse that is trying to throw the rider off, which is not uncommon in riding. The headquarters can be managed in several ways. Beginner riders are first introduced to the passive seat. As the name suggests, it is a way of riding in which the fluidity of the horse's movements is followed by the seat. The goal is not to hinder the movement of the horse, but to let it lead, while the rider follows it (Horse listening, 2022). The advantage of this seat is that it allows the horse to move more freely, almost as if there is no rider on the back. Then there is the restraining seat used by more experienced mostly dressage riders. This seat works by stopping the lower back and sometimes the upper leg in the middle of the horse's movement so that the rider signals to the horse a change in speed and pace. It is most often used when slowing down a horse. When the horse accelerates, the position of the seat changes forward, the body leans forward, and the legs are behind. This is how the rider signals the horse when he wants to transition from canter to gallop (Horse listening, 2022).

In addition to the seat and bridle, communication with the horse during riding is also done by pressing the calf or foot. For the rider to properly use his calves when riding, it is necessary to have a ridden horse on which he does not need to use his legs to maintain balance or hold on to it, that is, it is necessary to have an independent seat. Consistency is the most important factor in improving riding, both for the rider and the horse. For the position of the legs to be correct, it is necessary to have a good posture in the seat. Sitting on the seat bones will ensure proper "falling" of the legs on both sides of the horse (Horse listening, 2022). The position is correct when it follows a straight imaginary line connecting the head, shoulders, hips and heels. The rider most often uses the leaves when he wants to:

- Encourage the horse to start
- Encourage the horse to go forward and to the side
- Encourage the horse to bend in the body
- Control the pace of riding.

By starting the horse into a gait, pressure is exerted by the lower inner part of the leg. Given that it is an animal that is sensitive to touch, this pressure does not have to be strong. In dressage riding, encouraging the horse to go to the side is done by a combination of pressure on the calf of one leg, contact with the reins and the seat (Horse listening, 2022).

Training methods that value positive interaction between horse and rider

Thousands of years of coexistence between humans and horses have resulted in numerous new insights into horse ethology, relationships within the herd, and horse well-being. Animal welfare is defined as the state in which an individual tries to cope with the environment. The term includes the physical and mental state of animals, including the term health. The ideal level of animal well-being is a state of overall physical and mental health in which the animal is in harmony with its environment, and the term health is not only the absence of disease or injury but the ability of animals to harmonize the functions of the organism with the immediate environment (Broom and Johnson, 2000).

Fraser et al. (1997) describe three overlapping ethical theses expressed by public opinion on the quality of life of farm animals. These are:

1. animals should live naturally with the development and use of their natural abilities;

2. animals should feel good and should be freed from prolonged states of pain and fear and other negative states;
3. animals should be provided with a satisfactory state of health and normal functioning of physiological and behavioural needs.

By studying ethology, herd relationships and horse welfare, a trainer like Pat Parelli have dedicated his life to studying the Natural horsemanship method of working with horses that he has described in several of his books. This graduation thesis includes the Natural horsemanship method in Parelli's way, Liberty training and Clicker training. The majority of the equestrian sports population uses classical methods of horse training, while modern methods are those with a more positive outlook on the future. They allow the rider to connect with the horse on a deeper level and a new level of trust and partnership. This way of working and playing with horses brings longer-lasting and better-quality results in the world of riding that respect the natural aetiology of horses.

Conclusion

Interactions between horse and rider that lead to a positive relationship are realized through a series of actions of the rider towards the horse and vice versa. Some of these procedures are feeding, grooming, riding and treatment. In order to understand horses and see the world from their perspective, it is necessary to know the basic needs of animals, their senses and the way of communicating in the herd. The need for food, water, movement and socialization must be fulfilled in order to have a happy and satisfied horse, but these are only the basic and fundamental requirements of the animal. It is necessary to know how a horse thinks and experiences the world around him. With proper work and training, horses show a desire and willingness to communicate with people, and the forced way is the worst for both partners. The rider around the horse should be calm, reliable and have the qualities of a reliable leader, while the horse should have confidence in its rider. Acquiring all of the above requires time, perseverance and a desire for education and advancement. Constant quality work and time dedicated to the animal is the only thing that will lead to quality and successful maintenance of a positive relationship between horse and rider.

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Позитивне интеракције између коња и јахача

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Сажетак

Циљ рада био је представити начин развоја позитивне интеракције између коња и јахача у тренингу. Интеракције између коња и јахача које доводе до позитивног односа остварују се кроз низ поступака човјека према коњу те обратно. На интеракцију између коња и јахача утјече много чимбеника; сједиште јахача, метода тренинга, одговарајућа опрема и познавање основних карактеристика етологије коња. Веза између човјека и коња утјеловљење је највеће повезаности човјека с неком животињом док је ултимативни циљ јахања постизање сједињења с коњем, односно неке врсте флуидности. Коњи уз правилан рад и тренинг показују жељу и вољу за комуникацију с људима, док је присила у раду с коњем је најгори за оба партнера. Јахач у близини коња треба имати одлике смиреног и поузданог вође, док коњ треба имати поуздање у свог јахача. Константан правилан рад и вријеме посвећено коњу довести ће до квалитетног и успјешног стварања те одржавања позитивног односа између коња и јахача.

Кључне ријечи: коњ, тренинг, позитивна интеракција

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Assessment of contamination with heavy metals in food of animal origin

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Abstract

Heavy metals such as arsenic, cadmium, lead, and mercury are natural chemical compounds. They are present in water, soil, and the atmosphere in different amounts, but their presence has also been found in food because of contamination during production and storage, which is not desirable due to the bioaccumulation of heavy metals in the human body and its harmful effects on human health. The aim of the work is to determine the amount of heavy metals in certain types of food of animal origin that are available on the market in Republic of Srpska and Bosnia and Herzegovina in order to assess the real risks to human health based on compliance with the maximum allowed amount according to the Ordinance on the maximum allowed amounts of individual contaminants in food. The amount of total arsenic was determined by the hydride formation method of atomic absorption spectrometry according to *BAS EN 14546*, mercury by the spectrophotometric method on the Advanced Mercury Analyzer AMA-254, and cadmium and lead by the graphite technique AAS according to *BAS EN 14084*. Based on the test results, it was established that there were no heavy metals in the analyzed food samples that exceed the maximum allowed amounts, so based on this fact it can be concluded that the contamination in products of animal origin on the market of Republic of Srpska and Bosnia and Herzegovina with heavy metals does not pose a risk to human health. However, given the number of tests and increasing environmental contamination and heavy metal toxicity, monitoring of lead, cadmium, mercury, and arsenic must be carried out continuously to protect human health.

Key words: heavy metal, contamination of food, human health

Introduction

Heavy metals such as arsenic, cadmium, lead, and mercury are present in natural chemical compounds. They are present in water, soil, and the atmosphere in different amounts, but their presence has also been found in food because of contamination during production and storage, which is not desirable due to the bioaccumulation of heavy metals in the human body and its harmful effects on human health (Mitra et al., 2022; Tchounwou et al., 2012). Where heavy metals levels are higher, there is greater absorption of arsenic by animals, plants, and generally there is more of it in the food of that region (Tchounwou, et al., 2012).

Due to the toxic effects of heavy metals, in order to protect human health, the highest permitted amounts of contaminants in human food are prescribed, taking into account the most sensitive population groups, such as children, the elderly and pregnant women (Regulation, 2021). Arsenic is a metalloid that reacts chemically to produce both metallic and non-metallic characteristics. Unlike inorganic trivalent arsenic (As^{3+}), arsenic bound to organic molecules (As^{5+}) and elemental arsenic are not toxic (Mudhoo et al., 2011). Inorganic arsenic compounds have been linked to various types of cancer, according to refer to literature data (NRC, 1999). Due to its presence in the environment, arsenic can be found in food. Meat, dairy products and cereals are the main sources of inorganic arsenic, while seafood, fruits and vegetables are the main sources of organic arsenic (arsenobetaine). Organic compounds of arsenic, e.g. methylarsenic and dimethylarsenic acid are found in much smaller quantities compared to inorganic compounds such as arsenite and arsenate (Emsley, 2005). Levels of arsenic found naturally in soil, air and water are usually low, but can vary depending on the area, developed industry, arable agricultural land and other environmental pollutants. Past use of arsenic-containing pesticides on food crops, as well as pollution from mining, fracking, and coal-fired power plants, may contribute to higher levels of arsenic in the environment. Fish and shellfish are the main sources of organic As compounds, making them the main dietary sources of total As (EFSA, 2014, Vasi-Raki et al., 2010). Cadmium pollution of the not only aquatic environment occurs due to soil erosion into sediments, toxic pollution, and absorption. Cadmium can make people sick if they consume toxic food, breathe toxic air or drink water containing the metal. Cadmium does not possess properties that are beneficial for plants, animals, and humans' growth and metabolic activity (Hayat et al., 2018). The number of servings that can be eaten, before the Tolerable Weekly Intake (TWI) for methylmercury is reached, depends on the methylmercury content of the fish. For toddler children and women of childbearing age, the benefits of eating fish should be achieved by increasing consumption of species low in methylmercury. Apart from limiting the intake of high-mercury fish/seafood species in the daily diet, in order to avoid regular exposure above the TWI, it is not possible to give general recommendations on fish consumption throughout Europe. To achieve the health benefits derived from fish/seafood consumption, each country should consider its own fish consumption pattern and carefully assess the risk of exceeding the TWI for methylmercury (EFSA, 2015).

Environmental scientists have been interested in the toxicity of lead (Pb) because of its harmful effects on humans, animals, and plants. Lead can exist in both organic and inorganic forms (Kumur et al., 2020). Both forms of Pb are toxic, however organic Pb-complexes are excessively toxic to biological systems compared to inorganic Pb (WHO, 2010).

With canned food, there is a potential risk of lead migrating from the canning material into the food. The simplest transfer of lead to humans is through dust from the soil that enters people's living spaces, but also into drinking water. Lead exposure is of concern because lead is carcinogenic (group "B") to humans (IARC, 2019).

Due to the possible presence of heavy metals in meat and meat products, as a result of intentional or accidental exposure of domestic animals during fattening, and contamination of raw materials and finished products during processing, distribution and storage, it is necessary to continuously monitor the occurrence of these substances (Pleadin and Kovačević, 2016).

The aim of the work is to determine the amount of heavy metals in certain types of food of animal origin that are available on the market in Republic of Srpska and Bosnia and Herzegovina in order to assess the real risks to human health based on compliance with the maximum allowed amount according to the Ordinance on the maximum allowed amounts of individual contaminants in food. The assessment of the conformity of the test results of heavy metals is carried out according to the Ordinance on the Maximum allowed amounts for Certain Contaminants in Food (Official gazette of the Republic of Srpska, no 32/21).

Materials and Methods

Material

The total number of official food samples received in the laboratory for testing heavy metals was as follows: 181 samples of meat and meat products, 13 samples of milk and milk products, 38 samples of fish and meat products. The laboratory conducted a total of 661 tests of heavy metals, of which: 181 analyzes of arsenic, 177 analyzes of lead, and 176 analyzes of cadmium in meat and meat products; in milk and milk products 5 lead analyses, 13 arsenic analyses were done, and in fish and fish products 38 lead analyses, 38 mercury analyzes and 33 cadmium analyses were done. All samples that were delivered fresh or frozen until analysis was stored at refrigerator temperature or at -20 °C. Before analysis, meat and fish samples were chopped into 2-3 cm thick pieces and homogenized, and the products were tempered to room temperature and homogenized after opening. The samples were collected from April 2022 to March 2023.

Methods

For the determination of arsenic (As), the HGAAS atomic absorption spectrophotometric method was applied according to BAS EN 14546 Food products - Determination of total arsenic by hydride generation atomic absorption (HGAAS) after dry ashing. An Agilent instrument (Model AA 240) with a steam generator was used in these studies. The amount of mercury (Hg) was determined by the spectrophotometric method on the Advanced Mercury Analyzer AMA-254, and the test results were expressed in mg/kg of wet weight. The amount of lead (Pb) was determined at on atomic absorption spectrophotometer Agilent Technologies 240Z AA with Zeeman correction (GFAAS), and the measured quantities are expressed in mg / kg after microwave digestion according to BAS EN 14084 Food products - Determination of trace elements - Determination of lead, cadmium, zinc, copper, and iron by atomic absorption spectrometry (AAS).

The assessment of the conformity of the test results of heavy metals was carried out according to the Ordinance on the Maximum allowed amounts for Certain Contaminants in Food (Official gazette of the Republic of Srpska, no 32/21). Categories of tested samples and maximum allowed amounts (Regulation, 2021) are shown in Table 1, Table 2, and Table 3.

Table 1. Maximum permitted amounts of arsenic and lead in milk and milk products

Heavy metals	Type of food	Maximum limit*(mg/kg)
Pb	Raw milk, heat-treated milk and milk for production of dairy products	0.020
As	Milk and milk products	0.1

*Maximum allowed amounts

Table 2. Maximum allowed amounts for arsenic, lead and cadmium in meat and meat products

Heavy metals	Type of food	Maximum limit (mg/kg)
Pb	Meat (except offal) of cattle, sheep, pigs and poultry	0.10
Cd	1. Meat (except offal) of cattle, sheep, pigs and poultry 2. Liver of cattle, sheep, pigs, poultry and horses Kidneys of cattle, sheep, pigs, poultry and horses	0.050 0.50 1.0
As	1.Meat of cattle, sheep, pigs and poultry, except offal 2.Beef, sheep, pig and poultry meat products 3. Offal of cattle, sheep, pigs and poultry and their products	0.1 0.3 0.5

Table 3. Maximum allowed amounts for mercury, lead and cadmium in fish and fish product

Heavy metals	Type of food	Maximum limit (mg/kg)
Pb	Fish muscle Cephalopods Shellfish Shells	0.3 0.3 0.5 1.5
Cd	1.Muscle meat of fish, except for the species specifically mentioned in 2.Tuna 3.Muscle meat of the following fish: anchovy (Engraulis species) swordfish (Xiphias gladius) sardines (Sardina pilchardus) 4.crustaceans 5.shellfish 6.cephalopods	0.050 0.10 0.25 0.50 1.0 1.0
Hg	1. Fish products and fish muscle	0.5 or 1.0 **

** according to the types of fish and fish products that are defined according to the Regulation (2021).

Results and Discussion

Table 4 shows the average test results, as well as the minimum and maximum values of heavy metals expressed in mg/kg in meat and products.

Table 4. Test results of total number of analysis for heavy metals in meat and products

Heavy metals	Total analysis	Number of samples with quantity below <LOQ ***	****Mean ±SD mg/kg	Min (mg/kg)	Max (mg/kg)
Arsenic	181	37	0.011±0.0044	0.005	0.030
Lead	177	74	0.018±0.0092	0.010	0.059
Cadmium	176	68	0.002±0.0031	0.001	0.030

***LOQ for As: <0.005 mg/kg; Pb: <0.010 mg/kg; Cd: <0.001 mg/kg
****mean heavy metals content in the total number of analysed samples ± standard deviation of the mean value

Average values for lead. cadmium and arsenic in meat and products were respectively 0.018±0.0092 mg/kg. 0.002±0.0031 mg/kg and 0.011±0.0044 mg/kg. In the case of meat and meat products, the highest proportion of samples (42%) below the LOQ was recorded for Pb. Table 5 shows the average test results. as well as the minimum and maximum values of arsenic (As). expressed in mg/kg in different types of meat and products and offal.

Table 5. Results of testing the amount of As in meat, meat products and offal

Type of food- Meat	Mean ±SD (mg/kg)	Min-Max (mg/kg)
Beef meat Pork meat Poultry meat	0.011±0.0045 0.010±0.0042 0.011±0.0032	0.005-0.030 0.005-0.022 0.006-0.015
Meat products	0.014±0.0047	0.010-0.022
Offal of cattle. sheep. pigs and poultry and their products	0.014±0.0050	0.010-0.017
Mechanically deboned meat	0.009±0.039	0.005-0.016

In 20% of the tested meat samples, the amount of arsenic was not quantified (< LOQ). The highest average value of arsenic was determined in meat products 0.014±0.0047 mg/kg and offal 0.014±0.0050 mg/kg, and the lowest in mechanically deboned meat 0.009±0.039 mg/kg. The highest amount of arsenic was found in imported beef (0.030 mg/kg). In a study conducted from October 2015 to April 2022, Pećanac et al. (2022) determined the highest average value of arsenic in meat products of 0.023±0.0457 mg/kg, which is higher than the amount determined in this paper. Also, the mentioned authors recorded average values of arsenic in pork of 0.014±0.0078 mg/kg, in beef 0.013±0.0073 mg/kg, in poultry 0.012±0.00761 mg/kg and in mechanically deboned meat 0.012±0 .0054 mg/kg, which is in accordance with the results obtained in this paper. Pećanac et al. (2022) found that the highest amount of arsenic in meat products was 0.205 mg/kg.

In broiler meat tissues (wet weight), Ghosh et al (2012) determined the highest concentration of arsenic (μg/kg) in skin (218.8 ± 11.7), followed by liver (102.1 ± 8.0), lung (96.3 ± 5.6), kidney (88.2 ± 7.5) and thigh muscle (67.8 ± 5.1). Arsenic in broiler meat was below the maximum tolerable limits for humans.

Table 6 shows the average test results, as well as the minimum and maximum values of cadmium (Cd), expressed in mg/kg in different types of meat, meat products, and offal.

Table 6. Overview of results for Cd in meat and offal

Type of food	Mean ± SD (mg/kg)	Min-Max (mg/kg)
Beef meat Poultry meat Pork meat	0.002±0.0008 0.001 0.001±0.0011	0.001-0.012 0.001 0.001-0.007
Offal - chicken liver	0.030	0.030
Mechanically deboned meat	0.001±0.0006	0.001-0.002

In 74 samples (38.43%), out of a total of 177 samples, the amount of cadmium was below the limit of quantification (≤0.001 mg/kg). The highest amount of cadmium was determined in the liver sample of 0.030 mg/kg, and the highest average value of cadmium determined in beef meat was 0.002±0.0008 mg/kg. The average value in pork was 0.001±0.0011, with a maximum recorded amount of 0.007 mg/kg. Amount of 0.001±0.0006 mg/kg was determined in mechanically deboned meat, and 0.001 mg/kg in poultry meat. Pećanac et al. (2016) determined the amount of cadmium below the limit of quantification (56.12%) in 133 samples out of a total of 237. The same authors recorded the highest amount of cadmium in pork meat (0.025 mg/kg), and in beef meat a lower amount of 0.010 mg/kg. In poultry meat, the highest determined amount of Cd was 0.008 mg/kg. The results obtained in this paper are not in accordance with the results of Pećanac et al. (2016). Mračević et al. (2017) determined the amount of cadmium in pork at 0.03 mg/kg, and in beef at 0.04 mg/kg, which is significantly higher than the results obtained in this paper. In pig liver, the mentioned authors determined 0.18 mg/kg of cadmium. Table 7 shows the average test results, as well as the minimum and maximum lead content values expressed in mg/kg in different types of meat, products and offal.

Table 7. Test results for Pb in meat, meat products and offal

Type of food	Mean ± SD (mg/kg)	Min-max (mg/kg)
Beef meat poultry meat pork meat	0.018±0.0107 0.015±0.007 0.018±0.0079	0.011-0.059 0.011-0.031 0.010-0.040
Offal (pork liver)	0.012	0.012
Mechanically deboned meat	0.022±0.0008	0.018-0.031

The highest average value of lead was determined in mechanically deboned meat (0.022±0.0008 mg/kg). and the lowest in poultry meat (0.015±0.007 mg/kg). The highest amount of lead was recorded in beef meat (0.059 mg/kg), and the lowest in pork meat (0.010 mg/kg) (Table 6). The most important heavy metals as pollutants of meat products are cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg), especially Pb and Cd, since their presence in meat products has been confirmed by numerous studies (Hoha et al., 2014). Hana et al. (2022) determined the following average levels of As, Cd, Cr, Cu, Hg, Ni and Pb respectively: 0.018, 0.002, 0.061, 0.801, 0.0038, 0.055 and 0.029 mg/kg wet weight respectively in fresh meat. The average values for As and Cd determined in this paper agree with the results of the mentioned authors, while regarding Pb our results are somewhat lower.

Table 8 shows the total number of analyzes for heavy metals in milk and milk products, as well as the minimum and maximum values expressed in mg/kg. An overview of the results for As and Pb in milk and products is presented in Table 9.

Table 8. Overview of the number of tests and the average value of As in milk and milk products

Heavy metals	Total analysis	Number of samples with quantity below <LOQ***	Mean ± SD mg/kg	Min (mg/kg)	Max (mg/kg)
Arsenic in milk	5	4	-	0.011	0.011
Arsenic in milk product	8	-	0.019±0.0095	0.012	0.038
Lead	5	5	-	-	-

***LOQ for As: <0.010 mg/kg; ***LOQ for Pb: <0.008 mg/kg

Raw milk can be contaminated with heavy metals during its production (Ziarati et al., 2018). In the analyzed samples of milk and milk products, lead was quantified in only one out of five milk samples, while the amount of lead was above the LOQ in milk products in all tested samples. In all analised milk samples, lead was not quantified, the amount was below the LOQ.

Table 9. Test results for arsenic in milk and products of milk

Milk and Dairy Products	Amount (mg/kg)	Min-Max (mg/kg)
Raw milk, heat-treated milk, and milk for production of dairy products	<LOQ***	-
Cream with a minimum of 10% milk fat	0.019	-
Cream (crust) that it contains at least 70% milk fat in dry matter	0.029	-
milk spread	0.038	-
Cheese	0.014±0.0015	min 0.012 – max 0.015

***LOQ for As <0.010 mg/kg

The average value of the amount of arsenic obtained by testing five cheeses was 0.014±0.0015 mg/kg, and the highest determined amount was 0.015 mg/kg (table 8). Tajkarimi et al. (2008) determined mean concentrations of lead in fresh milk varying from 1.5 to 23.4 ng/L, in regions of Iran. Sola-Larrañaga and Navarro-Blasco (2009) determined the amount of lead in fresh milk which ranged from 0.55 to 18.7 µg/L, thus confirming the quantification of lead in milk. Mendil (2006) determined the amount of lead in different types of cheese in Turkey, whose values varied from 0.140 to 1.1 mg/kg.

Table 10 shows the total number of analysis for heavy metals in fish and fish products, as well as the minimum and maximum values expressed in mg/kg. An overview of the results for Hg in fish and fish products is presented in Table 11. Our analyzes of fish and fish products have shown that the content of Pb, Cd and Hg was significantly below the maximum allowed limits.

Table 10. Test results of total number of analysis for heavy metals in fish and products

Heavy metals	Total analysis	Number of samples with quantity below <LOQ***	Mean ± SD mg/kg	Min	Max
Pb	31	5	0.042±0.0221	0.010	0.313
Cd	31	-	0.028±0.0769	0.001	0.433
Hg	36	-	0.036±0.033	0.004	0.176

***LOQ for Pb <0.010 mg/kg

Table 11. Test results for Hg in the fish and fish product

Type of food	Mean ± SD (mg/kg)	Min-Max (mg/kg)
Hake	0.028±0.0298	0.020-0.061
Tuna	0.065±0.0581	0.009-0.176
Mackerel	0.031±0.0117	0.020-0.045
Sardine	0.035±0.0334	0.006-0.069
Herring	0.029±0.0255	0.004-0.052
Carp	0.025±0.0224	0.005-0.059
Mussels	0.007	0.007
Squids	0.015±0.009	0.006-0.024

In this paper, the lowest amount of mercury was recorded in herring (0.004 mg/kg). The average amount of mercury in fish and fish products was 0.036±0.033 mg/kg, and the highest amount of mercury 0.176 m/kg was determined in tuna meat, which is in accordance with the results of Pećanac et al. (2017), who examined 79 fish samples in their research, and the highest amounts of mercury were found in sea bass (0.230 mg/kg), tuna and monkfish (0.160 mg/kg) and sea bream (0.138 mg/kg). Pećanac et al. (2014) determined higher amounts of mercury, but still less than the maximum allowed amount in tuna in vegetable oil 0.256 mg/kg and canned tuna in pieces 0.229 mg/kg, which is higher than the amounts determined in tuna in this paper. The mentioned authors found the lowest amounts of mercury in fresh trout meat and frozen pangasius (0.005 mg/kg), and slightly more in fresh carp (0.006 mg/kg), which is lower than the amount of mercury determined in this work for carp.

Out of a total of 38 fish and product samples tested, in five samples the amount of lead was below the limit of quantification (<0.010 mg/kg). The average amount of lead in fish and fish products was 0.042±0.0221 mg/kg, and the highest amount of lead, according to the types of samples tested for lead, was recorded in sardines in tomato sauce 0.313 mg/kg. The highest amount of Cd was recorded in mussels (0.433 mg/kg), and the average amount of Cd in fish and products was 0.028±0.0769 mg/kg. Vitošević et al. (2007) examined the amounts of Cd, Pb and As in fish and products. The average amount of lead (0.0373±0.0176 mg/kg) agrees with our results, while the average amount of cadmium is significantly higher and does not agree with our results. Malavolti et al. (2022) examined the amount of lead in food and among other foods recorded the most lead in crustaceans and molluscs (0.039 mg/kg), which is not in agreement with our results.

Conclusion

Based on the obtained results, it was determined that the amounts of analyzed heavy metals in the food samples of animal origin (meat, milk, fish, and products), from the market of Republic of Srpska and Bosnia and Herzegovina, that was the subject of the testing in this work was not higher than the maximum allowed amount, thus complying with the requirements of the regulation. Based on this fact it can be concluded that the contamination of food with heavy metals does not pose a risk to human health in this region. However, given the number of tests and increasing environmental contamination and heavy metal toxicity, monitoring of lead, cadmium, mercury, and arsenic must be carried out continuously to protect human health.

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Evaluation of the microbiological suitability of drinking water on farms of domestic animals

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Abstract

The production and distribution of biological stable drinking water should be a non-negotiable goal for water utilities with the perspective of providing the same water quality to consumers than produced at the treatment facility. The microbiological quality of water is commonly defined as a maximum acceptable number or concentration of bacteria that do not constitute a health hazard. Many infectious diseases of animals and humans are transmitted by water contaminated with human and animal excrement, which becomes a source of pathogenic bacteria, viruses and parasites capable of surviving for different periods. The experiment used drinking water samples originating from farms of domestic animals from the territory of Republic of Srpska (Bosnia and Herzegovina) sampled in the Year 2021. A total of 117 samples were examined. The aim of this study is to determine the microbiological status of drinking water for animals in the Republic of Srpska (Bosnia and Herzegovina).

For microbiological testing of sterilized milk were used methods BAS EN ISO 6222, BAS EN ISO 7899-2 and BAS EN ISO 9308-1/A1. The microbiological state of water on farms in Republic of Srpska shows significant variations from year to year, and is still quite unfavorable considering the large number of unsatisfactory samples. This is particularly worrying considering the fact that over a third of farms are supplied with water from wells, which are not under constant sanitary supervision. Bearing this in mind, as well as the results of conducted research, it is necessary to comprehensively, regularly and routinely examine water samples originating from farms and take preventive measures in order to prevent water contamination, especially by animals.

Key words: drinking water, domestic animals, farms, microbiology

Introduction

The production and distribution of biological stable drinking water should be a non-negotiable goal for water utilities with the perspective of providing the same water quality to consumers than produced at the treatment facility. This can only be achieved by adequate monitoring and control of microbial processes during water treatment and distribution (Prest et al., 2016).

Water is essential for life, and a satisfactory (adequate, safe and accessible) supply must be available to all. Improving access to safe drinking-water can result in tangible benefits to health. Therefore, every effort should be made to achieve a drinking-water quality that is as high as possible (World Health Organisation, 2008).

Water temperature is an essential factor influencing bacterial growth kinetics and competition processes. Drinking water temperatures typically range between 3 and 25°C in European countries (Niquette et al., 2001; Uhl & Schaule, 2004), and fluctuate seasonally within this temperature range even within a single drinking water distribution system. Elevated water temperatures have often been associated with increased bacterial abundance in drinking water distribution systems (Francisque et al., 2009; Liu et al., 2013), and with higher numbers in indicator organisms such as coliforms or Aeromonas. In addition, water temperature can also affect bacterial community composition, by providing competitive advantages to specific bacterial species in defined temperature ranges, including pathogenic species (Vital et al., 2007; Vital et al., 2012). Vital et al. (2012) showed that the maximum growth rate and competitive fitness of *E. coli* grown with an indigenous drinking water community increased with temperature in the range of 12-30°C. There is therefore increased chance for problems associated with bacterial growth in summer periods (with higher water temperatures), such as hygienic risks, deterioration of aesthetic aspects of water, malfunctioning of water installations and exceeding of legal guidelines.

The microbiological quality of water is commonly defined as a maximum acceptable number or concentration of bacteria that do not constitute a health hazard (Directive (EU) 2020/2184 of the European Parliament and of the Council on the quality of water intended for human consumption (recast) [Directive], 2020). *E. coli*, intestinal enterococci, coliform bacteria and colony count on 22°C shall be monitored in accordance with the monitoring frequencies. *E. coli* and intestinal enterococci are considered "core parameters". Minimum requirements for parametric values used to assess the quality of water intended for human consumption is 0/100 ml for *E. coli* and intestinal enterococci. In Republic of Srpska (Bosnia and Herzegovina) the limit value for total count on 22°C (TC 22°C) is 100 CFU/ml and for total count on 37°C (TC

37°C) the limit is 20 CFU/ml. Also, coliform bacteria (CB), *Escherichia coli (E. coli)* and intestinal enterococci (EC) must not be detectable in 100 ml sample of water (Rulebook on the health safety of water intended for human consumption [Rulebook], 2017).

Many infectious diseases of animals and humans are transmitted by water contaminated with human and animal excrement, which becomes a source of pathogenic bacteria, viruses and parasites capable of surviving for different periods, and raise the health risk for many people throughout the world. In order to eliminate the risk related to disease transfer, water intended for mass consumption is treated and disinfected before use. Monitoring of water sources involves the determination of important microbiological and physico-chemical parameters which indicate first of all potential organic pollution, particularly pollution originating from animal excrement, storage of waste, natural and artificial fertilisers, and others (Sasakova et al., 2013; Fridrich et al., 2014). On the basis of the results, adequate measures can be taken that include prevention of contamination and systemic disinfection.

The use of indicator organisms, in particular the coliform group, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health. These are based upon the principle of the detection of selected bacteria that are indicative of either contamination or deterioration of water quality through the use of simple bacteriological tests. Indicator organisms are used to assess the microbiological quality of water. Whilst the presence of coliform bacteria does not always indicate a public health threat, their detection is a useful indication that treatment operations should be investigated (Edberg, 2000).

The use of indicator bacteria, in particular *Escherichia coli* (*E. coli*) and the coliform bacteria, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health (Hijnen et al., 2000).

The key criteria for ideal bacterial indicators of faecal pollution are that they should be universally present in large numbers in the faeces of human and other warm-blooded animals. They should also be present in sewage effluent, be readily detectable by simple methods and should not grow in natural waters. Ideally, they should also be of exclusive faecal origin and be present in greater numbers than faecally transmitted pathogens. No single indicator organism fulfils all these criteria, but the member of the coliform group that satisfies most of the criteria for the ideal indicator organism in temperate climates is *E. coli*. The presence of *E. coli* in a sample of drinking water may indicate the presence of intestinal pathogens. However, the absence of *E. coli* cannot be taken as an absolute indication that intestinal pathogens are also absent. *E. coli* bacteria are the only biotype of the family Enterobacteriaceae which can be considered as being exclusively faecal in origin (Edberg, 2000; World Health Organisation [WHO], 2008) and it can represent up to 95 % of the *Enterobacteriaceae* found in faeces.

Coliform bacteria belong to the family *Enterobacteriaceae* and share similar cultural characteristics. When coliform bacteria are isolated from drinking water supplies it is often useful to determine which species of coliform bacteria are present, particularly if problems recur, in order to determine the source and significance of the coliform bacteria being recovered. *E. coli* is a coliform bacteria and has historically been regarded as the primary indicator of faecal contamination of both treated and untreated water. *E. coli* occurs in the faeces of all mammals, often in high numbers (up to 10^9 per gram of faeces). This widespread faecal occurrence, coupled with methods that for the recovery and enumeration of *E. coli* are relatively simple to conduct, has contributed to the detection of this bacteria as the cornerstone of microbiological water quality assessment for over 100 years (Edberg, 2000; WHO, 2008).

Enterococci include a number of species that occur in the faeces of humans and warmblooded animals. The main reason for their enumeration is to assess the significance of the presence of coliform bacteria in the absence of *E. coli*, or to provide additional information when assessing the extent of possible faecal contamination. As such, they are regarded as secondary indicators of faecal pollution. Enterococci of faecal origin rarely multiply in water and are more resistant to environmental stress and chlorination than *E. coli* and coliform bacteria (WHO, 2008).

The results of the study of LeJeune et al. (2001) demonstrate that drinking water offered to cattle is often of poor microbiological quality. The extent of bacterial contamination observed in the drinking water offered to cattle demonstrates that the animals' daily exposure to *E. coli* from this source alone can be substantial. The association between the water quality parameters and the ecological factors measured suggest that many of the same factors that influence the survival and proliferation of bacteria in natural aquatic ecosystems have parallels in cattle water troughs. Multiple factors that influence the survival and persistence of bacteria in natural aquatic systems also appear to have an effect on the complex ecosystems present in cattle water troughs. Water troughs are a major source of exposure of cattle to enteric bacteria, including a number of foodborne pathogens, and this degree of bacterial contamination appeared to be associated with potentially controllable factors.

Safe drinking water for all is one of the major challenges of the 21st century and that microbiological control of drinking water should be the norm everywhere (Cabral, 2010). Routine basic microbiological analysis of drinking water should be carried out by assaying the presence of *E. coli* by culture methods.

The territorial and economic organization of the Republic of Srpska is conditionally created at the level of six regions: Banja Luka, Prijedor, Doboj, Bijeljina, East Sarajevo and Trebinje. The distribution of water is such that it is not enough where it is most needed (in the northern, most developed part of Republic of Srpska), and flows are most scarce during periods of the year when needs are greatest and when water quality protection problems are most serious.

In a study that included microbiological analysis of water on farms in the Republic of Srpska, Kalaba et al. (2015) found that 62.66% of water samples from animal farms were unsatisfactory and that the most common cause of water malfunction was enterococci, *E. coli* and total count. Kalaba et al. (2020) found that 26.20% of water samples from animal farms were unsatisfactory in the Republic of Srpska for the period 2015-2017. Also, they found that there were 63.40% of unsatisfactory samples due to the increased total count on 22°C and 54.90% due to the increased total count on 37°C, 58.80% due to the presence of intestinal enterococci, 31.40% due to coliform and 19% because *E. coli*. The lowest risk of the presence of coliforms and *E. coli* is in water supply system, and it is significantly higher in well water system.

In Croatia, two studies were conducted in which drinking water was analyzed from different farms (chicken broiler and laying hen farms, cattle and swine farms), where 40% of unsatisfactory samples was found (Denžić Lugomer et al., 2019) i.e. 20% (Kiš et al., 2017).

The aim of this study is to determine the microbiological status of drinking water for animals in the Republic of Srpska (Bosnia and Herzegovina).

Material and Methods

The experiment used drinking water samples originating from farms of domestic animals from the territory of Republika Srpska (Bosnia and Herzegovina) sampled in the Year 2021. A total of 117 samples were examined. Laboratory testing of samples was performed at the Public Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka.

Microbiological examination was carried out according to the Rulebook (2017). This included enumeration of colony forming units (CFU) expressed as total count of bacteria cultivated at 22°C (TC 22°C) and 37°C (TC 37°C) according to BAS EN ISO 6222 (Institute for standardization of Bosnia and Herzegovina [ISBIH], 2003), coliform bacteria (CB) and *E. coli* according to BAS EN ISO 9308-1/A1 (ISBIH, 2018) and intestinal enterococci (EC) according to BAS EN ISO 7899-2 (ISBIH, 2003).

In our research and in the statistical analysis of the obtained results, we used, as basic statistical methods, descriptive statistical parameters. The research results are presented in tables and figures.

Results and Discussion

No source of water that is intended for human consumption can be assumed to be free from pollution. All sources have different microbiological qualities and may be subject to natural or manufactured sources of pollution that may result in the deterioration of water quality to the point where treatment is no longer effective in removing all of the contamination. Zero-probability level of microbiological contamination of drinking water does not exist (Directive, 2020). It is incorrect to state that drinking water distribution and delivery systems should be sterile, the active growth of microorganisms is considered indicative of failures in water processing units or distribution. Adapted bacteria can grow even in oligotrophic systems, such as distilled water (Gottschal, 1992).

When it comes to the representation of samples in relation to the category, 61.54% of samples was from water supply system and 38.46% from wells.

Figure. 1 shows the regional representation of samples in % for the observed period, and Fig. 2 shows the regional representation of samples in % in relation to the category.

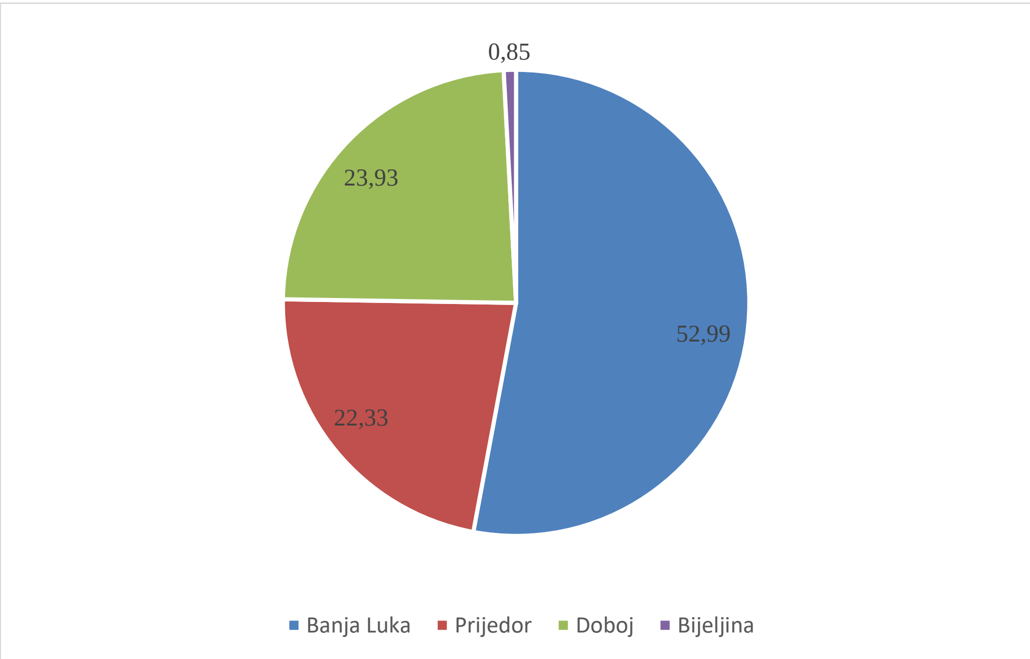

Figure 1. Regional representation of samples in % for the observed period

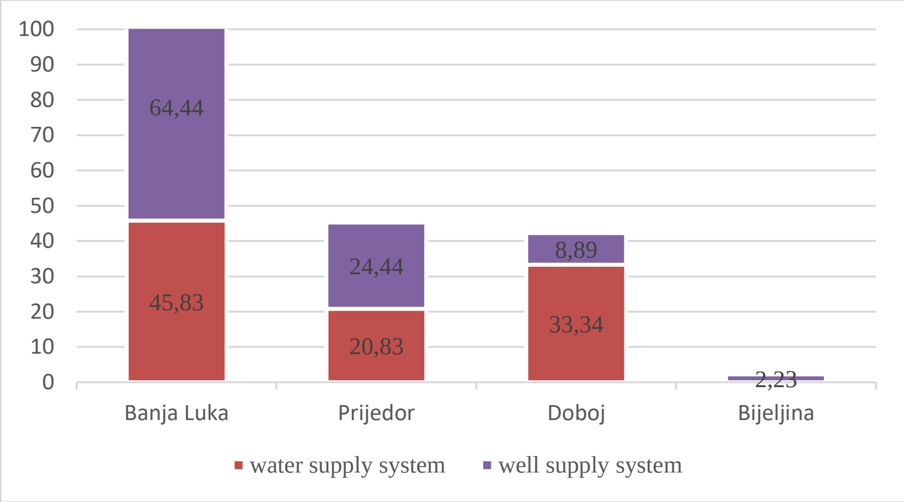

Figure 2. Regional representation of samples in % in relation to the category

Analysis of the testing samples taking into account the region and category indicates that most samples come from three regions (Banja Luka, Prijedor, Doboj). The analyzed number of samples originating from the region of Bijeljina is negligibly small, so it was left out of the discussion, while none of the samples analyzed from the region of East Sarajevo and Trebinje. Of the total number of tested samples, 69.23% were satisfactory, and 30.77% were unsatisfactory.

Table. 1 shows the test results by the category.

Table 1. Test results by category in %

Year	Satisfactory	Unsatisfactory
Water supply system	90.28	9.72
Well supply system	35.56	64.44

The obtained results for water supply system differ significantly and are better than results of Golić et al. (2021), but they are less favorable by well supply system since in our research there are twice as many unsatisfactory results. The obtained results differ from the results of Kalaba et al. (2015) for water supply system, who state that 30.50% of samples originating from water supply system are unsatisfactory, and thay are in accordance for well supply system where is 76.72% of samples are unsatisfactory.

Table. 2 shows the test results by region in %, and Tab. 3 shows the results by region in % in relation to categories.

Table 2. Test results by region in %

Region	Satisfactory	Unsatisfactory
Banja Luka	59.68	40.32
Prijedor	73.08	26.92
Doboj	89.29	10.71
Bijeljina	-	100

The obtained results are in accordance with the results of Kalaba et al. (2015) and Golić et al. (2021b), who found that the largest number of unsatisfactory samples were from the region Prijedor and Banja Luka, and that the significantly more favorable microbiological status of drinking water was in the region Doboj.

Table 3. Test results by region in % in relation to categories

Region	Water supply system		Well supply system	
	Satisfactory	Unsatisfactory	Satisfactory	Unsatisfactory
Banja Luka	93.93	6.06	20.69	79.31
Prijedor	80.00	20.00	63.64	36.36
Doboj	91.67	8.33	75.00	25.00
Bijeljina	-	-	0	100

The obtained results are in accordance with the results of similar studies (Denžić Lugomer et al., 2019; Kiš et al., 2017; Kalaba et al., 2015; Golić et al., 2021b). All types of water sources may be subjected to contamination by agricultural activity. Freerange animals may excrete faeces into water, and animals like cattle have a habit of wading into water and stirring up sediments. Rainfall can result in the run-off of faecal matter from agricultural and other rural lands into rivers, lakes, reservoirs and springs. The discharge of effluents from sewage treatment works, septic tanks and cesspools can dramatically increase the microbial content of surface waters. The potential source of coliform bacteria in water supplies result from sub-optimal operation of water treatment processes or ingress of contamination from breaches in the integrity of the distribution system. These include for example, leaking hatches on service reservoirs, contamination via air-valves and stop valves, infiltration into mains and service reservoirs, cross connections and back-flow effects. Coliform bacteria can be present in domestic plumbing systems with kitchen taps and sinks being recognised sources of these organisms. There are large differences between water and quality control systems for water destined for human or animal consumption (Eenige et al., 2013). For example, testing for microorganisms occurs less frequently in cattle systems; substrates for bacteria are often present in cattle systems; few cattle systems are screened for faecal contamination, even though this is a major source of contaminants; in many cattle systems water can flow in more than one direction, which is not the case for human water systems; the existence and implementation of cleaning and disinfection protocols are poor in cattle systems and biofilms are more often present in the pipelines of cattle systems. The latter phenomenon often leads to the presence of many different bacteria in cattle drinking systems.

Comparing the results of water testing in relation to the category, it can be noticed that there is a significantly higher number of unsatisfactory samples of well water in relation to water supply sistem, which is expected considering that the public water supply system is under daily control with regular chlorination. In contrast, well water supplies one or fewer farms, is not under constant control but very rarely, most often once a year as an official control, or in the event of an animal health incident. Also, well waters are not flowing but stagnant, so the microbiological status of this water is greatly influenced by the number of animals drinking from the well, i.e. the speed and amount of water consumption from the well.

Table 4. show unsatisfactory results in % according to test parameter.

Table 4. Unsatisfactory test results in % according to test parameter

Test parameter				
TC 22°C	TC 37°C	EC	*E. coli*	CB
19.66	22.22	17.95	23.08	21.37

Table 5. shows the unsatisfactory test results of samples by region in % in relation to the test parameter.

Table 5. Unsatisfactory test results of samples by regions in % in relation to the test parameter

Region	Test parameter				
	TC 22°C	TC 37°C	EC	*E. coli*	CB
Banja Luka	24.19	27.42	25.81	32.26	33.87
Prijedor	15.38	15.38	11.54	11.54	11.54
Doboj	7.14	10.71	-	7.14	3.57
Bijeljina	100	100	100	100	100

According to the WHO (2008), *E. coli* are the only true indicator of faecal contamination and they are exclusively of intestinal origin and are found in faeces. Their presence indicates mostly fresh faecal contamination and thus points to serious shortcomings in protection of the specific water source, treatment of water and its hygienic safety. Faecal streptococci represent evidence of faecal contamination and tend to persist for longer in the environment than thermotolerant or total coliforms. Colony counts are enumerations of the general population of heterotrophic bacteria present in water supplies. The enumerations may represent bacteria whose natural habitat is the water environment or those that have originated from soil or vegetation. The heterotrophic plate count includes all of the microorganisms that are capable of growing in or on a nutrient-rich solid agar. Two incubation temperatures and times are used for total count, 37°C for 48 h to encourage the growth of bacteria of mammalian origin, and 22°C for 72 h to enumerate bacteria that are derived principally from environmental sources. The test results indicate that the causes of microbiological water malfunction come from animals, having in mind that the largest number of unsatisfactory samples is due to the increased total count at 37°C, but we should not ignore the fact of the possibility of contamination from the environment due to the increased total count at 22°C. Contamination from animals is very possible due to inadequate drainage of waste and fecal water and consequent contamination of groundwater, because wells are usually not planned and are mostly located near farms. The obtained results are in accordance with the results Kalaba et al. (2015), Kalaba et al. (2020) and Golić et al. (2021a) and indicate significant fecal contamination of water, especially with *E. coli* and coliforms. In relation to the results of Golić et al. (2021a) and Golić et al. (2021b) for the period 2018-2020, there was a significant deterioration in terms of *E. coli* and coliforms. The presence of pathogenic bacteria from water supply system is a particularly worrying fact given that water must be microbiologically correct, which means that it must not contain pathogens (Rulebook, 2017). A possible explanation for this is dilapidation and damage to water supply installations in farms leading to water contamination. This is in line with observations Interact (2006), which indicate that it is the microbiological quality of drinking water for cattle diminishes once the water has entered the farm. A field survey in the Netherlands has pointed out that at the entry point (130 dairy farms; 285 samples) 98% of water was of suitable microbiological quality, but at the end point (199 samples) this percentage had dropped to 60%, so that 40% of the water samples were unsuitable as drinking water for cattle. For this three-year period, there were on average 76.59±1.89% satisfactory and 23.41±1.89% unsatisfactory samples. The obtained results, in contrast to the results of Kalaba et al. (2015), indicate a much better microbiological status of drinking water on animal farms and are in accordance with the results of Kalaba et al. (2020).

All types of water sources may be subjected to contamination by agricultural activity (Environment Agency [EA], 1998). Freerange animals may excrete faeces into water, and animals like cattle have a habit of wading into water and stirring up sediments. Rainfall can result in the run-off of faecal matter from agricultural and other rural lands into rivers, lakes, reservoirs and springs. The discharge of effluents from sewage treatment works, septic tanks and cesspools can dramatically increase the microbial content of surface waters. The potential source of coliform bacteria in water supplies result from sub-optimal operation of water treatment processes or ingress of contamination from breaches in the integrity of the distribution system. These include for example, leaking hatches on service reservoirs, contamination via air-valves and stop valves, infiltration into mains and service reservoirs, cross connections and back-flow effects. Coliform bacteria can be present in domestic plumbing systems with kitchen taps and sinks being recognised sources of these organisms.

According to the WHO (2008), *E. coli* are the only true indicator of faecal contamination; they are exclusively of intestinal origin and are found in faeces. Their presence indicates mostly fresh faecal contamination and thus points to serious shortcomings in protection of the specific water source, treatment of water and its hygienic safety. Faecal streptococci represent evidence of faecal contamination and tend to persist for longer in the environment than thermotolerant or total coliforms. The obtained results indicate significant fecal contamination of water, especially with intestinal enterococci and coliforms, and less with *E. coli*.

Colony counts are enumerations of the general population of heterotrophic bacteria present in water supplies. The enumerations may represent bacteria whose natural habitat is the water environment or those that have originated from soil or vegetation. The heterotrophic plate count includes all of the microorganisms that are capable of growing in or on a nutrient-rich solid agar. Two incubation temperatures and times are used for total count, 37°C for 48 h to encourage the growth of bacteria of mammalian origin, and 22°C for 72 h to enumerate bacteria that are derived principally from environmental sources (EA, 1998). The test results indicate that the causes of microbiological water malfunction come from animals, having in mind that the largest number of unsatisfactory samples is due to the increased total count at 37°C, but we should not ignore the fact of the possibility of contamination from the environment due to the increased total count at 22°C. Contamination from animals is very possible due to inadequate drainage of waste and fecal water and consequent contamination of groundwater, because wells are usually not planned and are mostly located near farms.

Kalaba et al. (2015) state that 30.50% of samples originating from water supply system and 76.72% of samples from wells are unsatisfactory. Compared to the period 2015-2017 (Kalaba et al., 2015; Kalaba et al, 2020), the level of pathogenic bacteria in drinking water is reduced, especially for intestinal enterococci and coliforms, as well as the total number of microorganisms at 22°C and 37°C. The obtained results indicate a significantly improved microbiological status of drinking water compared to the results Jaki et al. (2010), Denžić et. al (2012) and in accordance with the results Denžić et al. (2013).

Table. 6 and 7 shows the unsatisfactory test results of water from water supply system and well water by region in % in relation to the test parameter.

Table 6. Unsatisfactory test results of water from water supply system by regions in % in relation to the test parameter

Region	Test parameter				
	TC 22°C	TC 37°C	EC	*E. coli*	CB
Banja Luka	-	3.03	3.03	6.06	6.06
Prijedor	13.33	6.67	-	-	-
Doboj	8.33	8.33	-	4.17	4.17

Table 7. Unsatisfactory test results of well water by regions in % in relation to the test parameter

Region	Test parameter				
	TC 22°C	TC 37°C	EC	*E. coli*	CB
Banja Luka	51.72	55.17	51.72	62.07	65.52
Prijedor	18.18	27.27	27.27	27.27	27.27
Doboj	-	25.00	-	25.00	-
Bijeljina	100	100	100	100	100

The obtained results for water supply system differ significantly and are better in relation to the results Denžić et al. (2013), while the results obtained for well water are in accordance with the results Denžić Lugomer et al. (2019) and Kiš et al. (2017). Contrary to this, the obtained results differ significantly and are less favorable than the results Golić et a. (2021a).

There are large differences between water and quality control systems for water destined for human or animal consumption (Eenige et al., 2013). For example, testing for microorganisms occurs less frequently in cattle systems; substrates for bacteria are often present in cattle systems; few cattle systems are screened for faecal contamination, even though this is a major source of contaminants; in many cattle systems water can flow in more than one direction, which is not the case for human water systems; the existence and implementation of cleaning and disinfection protocols are poor in cattle systems and biofilms are more often present in the pipelines of cattle systems. The latter phenomenon often leads to the presence of many different bacteria in cattle drinking systems.

Comparing the results of water testing in relation to the category, it can be noticed that there is a significantly higher number of unsatisfactory samples of well water in relation to water supply sistem, which is expected considering that the public water supply system is under daily control with regular chlorination. In contrast, well water supplies one or fewer farms, is not under constant control but very rarely, most often once a year as an official control, or in the event of an animal health incident. Also, well waters are not flowing but stagnant, so the microbiological status of this water is greatly influenced by the number of animals drinking from the well, i.e. the speed and amount of water consumption from the well.

It is incorrect to state that drinking water distribution and delivery systems should be sterile, the active growth of microorganisms is considered indicative of failures in water processing units or distribution. Adapted bacteria can grow even in oligotrophic systems, such as distilled water (Gottschal, 1992; Poindexter, 1987; Roszak & Colwell, 1987).

The presence of pathogenic bacteria from water supply system is a particularly worrying fact given that water must be microbiologically correct, which means that it must not contain pathogens (Rulebook, 2017). A possible explanation for this is dilapidation and damage to water supply installations in farms leading to water contamination. This is in line with observations Interact (2006), which indicate that it is the microbiological quality of drinking water for cattle diminishes once the water has entered the farm. A field survey in the Netherlands has pointed out that at the entry point (130 dairy farms; 285 samples) 98% of water was of suitable microbiological quality, but at the end point (199 samples) this percentage had dropped to 60%, so that 40% of the water samples were unsuitable as drinking water for cattle.

Conclusion

The microbiological state of water on farms in Republic of Srpska (Bosnia and Herzegovina) shows significant variations from year to year, and is still quite unfavorable considering the large number of unsatisfactory samples. This is particularly worrying considering the fact that over a third of farms are supplied with water from wells, which are not under constant sanitary supervision. Bearing this in mind, as well as the results of conducted research, it is necessary to comprehensively, regularly and routinely examine water samples originating from farms and take preventive measures in order to prevent water contamination, especially by animals.

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