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## NUTRITIVE VALUE OF FIELD PEA – OAT AND COMMON VETCH – OAT BICROPS

**Jordan P. Marković<sup>1\*</sup>, Jasmina R. Milenković<sup>1</sup>, Mirjana P. Petrović<sup>1</sup>,  
Vladimir G. Zornić<sup>1</sup>, Nedeljko Lj. Racić<sup>1</sup>,  
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**Abstract:** Peas and vetches are the most important plant species for the production of forages, where they can be used as hay, haylage or silage. Due to their specific characteristics – tendency to lodging and high buffering capacity, they are usually grown in a mixture with small grains as support crops to prevent or reduce lodging, reduce buffering capacity, and at the same time obtain biomass suitable for the ensiling process. In pure crops, peas are most often used as green feeds or hay at the stage of first pod formation, while the best quality of vetch dry matter has been achieved by using it at the stage of full flowering or the beginning of first pod formation, when up to 25% of crude protein is obtained in the biomass of pure culture. The nutritive value of pure crops of peas, common vetch and oats, and their mixtures is presented as a function of the structure of the mixtures and the stage of use. Special attention is paid to the content of cell wall components and the dry matter digestibility of these feeds. Because of all of these mentioned above, it is particularly important to choose the right harvesting time to balance the yield and nutritional value of feeds used in bicrops.

**Key words:** pea – oat mixture, common vetch – oat mixtures, nutritive value.

### Introduction

Modern livestock production implies intensive breeding of specific domestic animal species selected for high genetic potential of animal production. The high price of the concentrated feeds indicates the fact that the economy of the animal production is primarily reflected in the maximum use of forages, which are significantly cheaper than concentrates. Based on the above, the preservation of annual legumes (peas and vetches) in combination with small grains by ensiling is

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one of the possible solutions for the production of high quality forages for ruminant nutrition.

Advances in dairy farming are primarily the result of significant advances in nutrition practice. The greatest progress has been made through the application of a continuous diet of preserved forages throughout the year (Radivojević et al., 2015). Alfalfa and corn are the main forage crops in Serbia and the Balkan region. However, climatic conditions during the summer season are less optimal for corn production, and high summer temperatures contribute to the production of corn silage with a higher lignin content, which is characterized by lower digestibility. There are few forage crops that can completely replace corn silage in the diet of dairy cattle. However, the solution to this problem is the silage of annual legumes grown in the bi – cropping system with small grains (Radivojević et al., 2015).

The nutritive value of grasses and legumes decreases as the plant matures, primarily due to the lower leaf to stem ratio in mature plants and the very rapid decline in stem quality during plant growth and development. To produce high quality forages, it is necessary to harvest these plants at a stage of development when the leaf to stem ratio is high. However, the chemical composition of annual legumes and cereals does not change in the same way as the chemical composition of grasses and perennial legumes, probably due to the ratio of pods to seeds in the DM (dry matter) (Salawu et al., 2001). Therefore, it is very difficult to determine when to harvest these crops to obtain high quality forages. On the other hand, the chemical composition of annual legumes – field peas and vetches – does not change as much as the chemical composition of cereals, so the harvesting time of these plants grown in bi – crop systems depends largely on the developmental stage of cereals (Salawu et al., 2001; Marković et al., 2019a, b).

Barley, oats and wheat are the cereals most commonly used in the bi – crop systems with leguminous plants (Chapko et al., 1991; Salawu et al., 2001). The type of cereals used in the bi – crop systems greatly affects the nutritive value and DM yield, but it should be noted that barley and oats have some advantages over the other cereals (Khorasani et al., 1993). Some authors (Chapko et al., 1991; Salawu et al., 2001) indicated that growing field peas in a bi – crop with wheat, oats and barley leads to an increase in CP (crude protein) content and a decrease in NDF (neutral detergent fiber) and ADF (acid detergent fiber) content compared to cereals grown in monoculture. Mustafa and Seguin (2004) also found that field pea silage is characterized by a higher CP content and lower NDF and ADF contents in relation to pea – cereal silages.

### **The importance of pea and vetch cultivation in bi – crop with oats**

Annual legumes are mostly grown as monocultures, but in some countries intercropping with cereals is a common practice (Klimek-Kopyra et al., 2014).

Field peas and vetches are important plant species for the production of forages used as hay, haylage or silage (Dinić et al., 2008, 2011). Due to their characteristics (tendency to lodge down and high buffer capacity), they are most often grown in a mixture with small grains as supporting crops to prevent or reduce lodging, reduce buffer capacity, and also produce biomass suitable for ensiling process (Đorđević et al., 2010; Blagojević et al., 2014; 2015a, b; 2017). In pure crops, peas are most often used as hay when the first pods are formed, whereas the best quality of common vetch is achieved by using it at full flowering or when the first pods start to form (Karagić et al., 2011a).

Field pea (*Pisum sativum* L.) is an exceptional plant species for animal nutrition, both from the point of view of its use as a cereal and as animal feed. Compared to other legumes of similar quality and uses, peas have much more modest requirements for growing conditions. Pea is also a species that easily lodges down, so it is sown in a mixture with cereals as supporting crops (Omokanye, 2014). As a pure crop, for green forage and hay, it is used at the stage of first pod formation. The herbage yield of peas varies depending on the variety, fertilization, harvest period and cultivation method. Ensiling is the best solution to preserve the nutritional value of pea herbage, until the time of use (Kwabiah, 2004).

Common vetch (*Vicia sativa* L.) is used for feeding animals in the form of green forage and hay, but also as silage (Karagić et al., 2011b; 2012). It is rarely sown as a pure crop, and due to its pronounced tendency to lodge down, it is most often grown in a mixture with small grains – mainly oats and rye, which increases the total herbage and dry matter yield as well as the nutritional value (Seven and Cerci, 2006). The best dry matter quality of vetches is achieved when they are harvested at the full flowering stage or at the formation of the first pods, when the dry matter contains from 18.6 to 22.6% crude protein (Caballero et al., 1995).

### **Green forage and dry matter yields of pea – oat and common vetch – oat mixtures**

When legumes are grown in a bi – crop system with small grains, the choice of leguminous species has a significant impact on forage yield and quality (Altin and Ucan, 1996). Furthermore, several factors influence forage yield in a mixed crop, including the variety, the proportion of seeds in the mixture, and the qualitative properties of the components in the mixture (Caballero et al., 1995; Carr et al., 2004; Drooshiotis, 1989). Numerous authors have noted that combining annual legumes and small grain crops results in higher yields and a more balanced nutrient ratio (Haymes and Lee, 1994; 1999; Anil et al., 1998; Carr et al. 2004).

The yield of DM and the content of CP in combined crops are primarily influenced by the proportion of the individual components in the mixture. The

proportion of leguminous seeds in the mixture (peas and vetches) can affect the CP content of the forage obtained, but has no effect on DM yields in combined crops of annual legumes and grains (Carr et al., 1998). The proportion of cereal seeds in the mixture, on the other hand, significantly affects DM yields.

According to Ericson and Norgren (2003), the DM yield of peas grown in a monoculture ranged from 3.5 to 6 t ha<sup>-1</sup>, whereas the DM yield of a combined crop of peas and oats ranged from 4.7 to 6.8 t ha<sup>-1</sup> when the proportion of pea seeds in the mixture was 330 to 460 g kg<sup>-1</sup>. Åman and Graham (1987) found comparable pea DM yields ranging from 3.8 to 6.5 t ha<sup>-1</sup>. Fraser et al. (2001) indicated that the pea DM yield was comparable at various stages of development, primarily due to an increase in the plant DM as the plant grows and develops.

Bayram and Celik (1999) indicated that the highest DM yield of 10.46 t ha<sup>-1</sup> was found in a mixture of 25% vetches and 75% oats, while the DM yield of a pure vetch crop was 9.65 t ha<sup>-1</sup>. Caballero et al. (1995) found that a combined vetch – oat bi – crop produced 34% more DM than a pure vetch crop, but 57% less than a pure oat crop. Basbag et al. (1999) determined that the proportion of small grains in the mixture increased the forage yield of combined crops, and that the highest DM yield of 7.6 t ha<sup>-1</sup> was established in a 50:50 mix of vetches and barley. Konak et al. (1997) reported that forage yield of a vetch – oat mixture was 41.14 t ha<sup>-1</sup>, and DM yield was 11.18 t ha<sup>-1</sup>. According to Tuna and Orak (2002), the highest herbage yield (29.0 t ha<sup>-1</sup>) was established in a mixture containing 25% vetches and 75% oats, and the lowest forage yield in a pure crop of vetches (17.8 t ha<sup>-1</sup>). The same authors indicated that the mixture containing 75% vetches and 25% oats produced the lowest forage yield (15.6 t ha<sup>-1</sup>), but the highest DM yield was obtained in the pure oat crop (7.2 t ha<sup>-1</sup>) and the lowest in the mixture with 75% vetches (5.0 t ha<sup>-1</sup>). Similar results were found by Altin and Ucan (1996), Bayram and Celik (1999), Basbag et al. (1999), Tansi et al. (1993).

The data on forage and DM yield in the study conducted by Marković et al. (2018a) presented in Tables 1 and 2 show statistically significant differences among the treatment means.

Table 1. The effect of vetch + oat pure stands and their mixtures on green forage yield (t ha<sup>-1</sup>) at different growth stages.

Mixture rate oat:vetch (%)	Growth stage I	Growth stage II	Growth stage III	Means
Pure oat	59.7 <sup>a</sup>	49.9 <sup>a</sup>	49.0 <sup>a</sup>	52.86 <sup>A</sup>
60:40	55.2 <sup>b</sup>	46.6 <sup>b</sup>	38.7 <sup>b</sup>	46.83 <sup>B</sup>
50:50	46.5 <sup>c</sup>	44.1 <sup>b</sup>	33.0 <sup>c</sup>	41.20 <sup>C</sup>
40:60	38.6 <sup>d</sup>	34.5 <sup>c</sup>	29.5 <sup>d</sup>	34.20 <sup>D</sup>
Pure vetch	29.2 <sup>e</sup>	22.8 <sup>d</sup>	20.0 <sup>e</sup>	24.00 <sup>E</sup>
Means	45.84 <sup>A</sup>	39.58 <sup>B</sup>	34.04 <sup>C</sup>	

Different letters denote significantly different means (P< 0.05), Marković et al. (2018a).

The maximum DM yield of 14.15 t ha<sup>-1</sup> was produced by oat monocultures (Table 2), followed by a mixture of 60% oats + 40% vetches (12.48 t ha<sup>-1</sup>). As the growth stage progressed, the DM yield increased from the beginning of vetch flowering to forming the first pods on 2/3 of the vetch plants. In the pure stand treatments, the highest DM yield of 14.68 t ha<sup>-1</sup> was produced by oats at the third growth stage, and the lowest by vetches in monoculture (5.81 t ha<sup>-1</sup>) at the same growth stage.

Table 2. The effect of pure vetch + oat stands and their mixtures on dry matter yield (t ha<sup>-1</sup>) at different growth stages.

Mixture rate oat:vetch (%)	Growth stage I	Growth stage II	Growth stage III	Means
Pure oat	13.82 <sup>a</sup>	13.97 <sup>a</sup>	14.68 <sup>a</sup>	14.15 <sup>A</sup>
60:40	13.13 <sup>a</sup>	12.81 <sup>b</sup>	11.50 <sup>b</sup>	12.48 <sup>B</sup>
50:50	11.06 <sup>b</sup>	12.12 <sup>b</sup>	9.86 <sup>c</sup>	11.01 <sup>C</sup>
40:60	9.56 <sup>c</sup>	10.60 <sup>c</sup>	9.06 <sup>c</sup>	9.74 <sup>D</sup>
Pure vetch	7.00 <sup>d</sup>	6.21 <sup>d</sup>	5.81 <sup>d</sup>	6.34 <sup>E</sup>
Means	10.91 <sup>A</sup>	11.14 <sup>A</sup>	10.18 <sup>B</sup>	

Different letters denote significantly different means (P< 0.05), Marković et al. (2018a).

### Nutritive value of pea – oat and common vetch – oat mixtures

It is important to mention that the most significant changes in the chemical composition of pea dry matter occur during the grain filling stage of development (Åman and Graham, 1987). Nutrients, especially CP and carbohydrates, are translocated from the vegetative parts of the plant to the seeds, while cellulose and hemicellulose, as well as lignin are accumulated in the leaf and especially in the stem. In general, during growth and development, the soluble sugars converted to starch and the content of structural carbohydrates are increased (Åman and Graham, 1987).

CP is one of the most expensive components in animal feeds, with a large impact on production costs. Feeding excess CP can result in unnecessary feed expenses with no return in milk or milk protein yield (Blagojević et al., 2017). Jaster et al. (1985) indicated that CP content and soluble protein content decreased with growth and development in the combined crop of peas and oats. In contrast, the dry matter content, starch and NDF of peas increase with the stage of development progress. The content of CP, starch, NDF, ADF and soluble sugars in combined bi – crop systems of peas and oats depends on the plant developmental stage of the individual components, even in bi – crop mixtures, and also on the seed ratio of the individual components in the mixtures (Salawu et al, 2001). Previous research has established that ruminants prefer legumes to grasses,

regardless of whether they have comparable DMD (Beever and Throp, 1996; Salawu et al., 2002).

Marković et al. (2017a) investigated the content of CP and protein fractions of pea:oat mixtures at two different mixture rates: 50% peas + 50% oats and 75% peas + 25% oats at three developmental stages: 10% of flowering, forming the first pods on 2/3 of pea plants and forming green seeds in 2/3 of pods. These authors found that the content of CP during plant growth and development ranged from 176.82 to 136.00 g kg<sup>-1</sup> DM in the 50:50 pea – oat mixture and from 192.58 to 166.80 g kg<sup>-1</sup> DM of the 75:25 pea – oat mixture, respectively. The same authors also indicated that the average level of CP in the 75:25 pea – oat mixture was 20.59 g kg<sup>-1</sup> DM higher than in the 50:50 pea – oat mixture, due to the higher quantity of peas in this mixture.

Table 3. The crude protein content and fraction distribution determined according to the Cornell Net Carbohydrate and Protein System (CNCPS).

	CP g kg <sup>-1</sup> DM	PA g kg <sup>-1</sup> CP	PB <sub>1</sub> g kg <sup>-1</sup> CP	PB <sub>2</sub> g kg <sup>-1</sup> CP	PB <sub>3</sub> g kg <sup>-1</sup> CP	PC g kg <sup>-1</sup> CP
R <sub>1</sub>	P <sub>1</sub> 176.82 <sup>b</sup>	473.70 <sup>c</sup>	79.72 <sup>a</sup>	279.06 <sup>c</sup>	82.87 <sup>a</sup>	84.65 <sup>d</sup>
	P <sub>2</sub> 152.58 <sup>d</sup>	549.85 <sup>b</sup>	17.98 <sup>d</sup>	276.02 <sup>d</sup>	52.50 <sup>e</sup>	103.65 <sup>a</sup>
	P <sub>3</sub> 136.00 <sup>e</sup>	500.60 <sup>d</sup>	11.97 <sup>e</sup>	375.60 <sup>a</sup>	15.23 <sup>f</sup>	96.60 <sup>b</sup>
R <sub>2</sub>	P <sub>1</sub> 192.58 <sup>a</sup>	535.55 <sup>c</sup>	9.78 <sup>e</sup>	350.37 <sup>b</sup>	37.65 <sup>d</sup>	66.65 <sup>f</sup>
	P <sub>2</sub> 167.06 <sup>c</sup>	559.70 <sup>a</sup>	33.10 <sup>c</sup>	267.05 <sup>e</sup>	63.75 <sup>b</sup>	76.40 <sup>e</sup>
	P <sub>3</sub> 166.80 <sup>c</sup>	459.80 <sup>f</sup>	51.20 <sup>b</sup>	374.85 <sup>a</sup>	20.65 <sup>e</sup>	93.50 <sup>c</sup>
Average R <sub>1</sub>	155.13 <sup>b</sup>	508.05 <sup>b</sup>	36.55 <sup>a</sup>	310.23 <sup>b</sup>	50.20 <sup>a</sup>	94.97 <sup>a</sup>
Average R <sub>2</sub>	175.72 <sup>a</sup>	518.35 <sup>a</sup>	31.37 <sup>b</sup>	330.76 <sup>a</sup>	40.67 <sup>b</sup>	78.85 <sup>b</sup>
Average P <sub>1</sub>	184.85 <sup>a</sup>	504.63 <sup>b</sup>	44.75 <sup>a</sup>	314.74 <sup>b</sup>	60.23 <sup>a</sup>	75.65 <sup>c</sup>
Average P <sub>2</sub>	160.03 <sup>b</sup>	554.78 <sup>a</sup>	25.54 <sup>c</sup>	271.53 <sup>c</sup>	58.12 <sup>b</sup>	90.03 <sup>b</sup>
Average P <sub>3</sub>	151.45 <sup>c</sup>	480.20 <sup>c</sup>	31.58 <sup>b</sup>	375.22 <sup>a</sup>	17.95 <sup>c</sup>	95.05 <sup>a</sup>

R<sub>1</sub> – first mixture, 50% peas + 50% oats; R<sub>2</sub> – second mixture, 75% peas + 25% oats; P<sub>1</sub> – a cutting of the biomass at the start of pea flowering (10% of flowering); P<sub>2</sub> – a cutting of biomass at forming the first pods on 2/3 of pea plants; P<sub>3</sub> – a cutting of biomass at forming of green seeds in 2/3 of pods; CP – crude protein, PA – non-protein nitrogen; PB<sub>1</sub> – the rapidly degraded crude protein; PB<sub>2</sub> – the intermediately degraded crude protein; PB<sub>3</sub> – the slowly degraded crude protein; PC – the bound crude protein; Different letters denote significantly different means (P< 0.05), Marković et al. (2017a).

An analysis of variance found statistically significant differences among mixtures for CP content in the study conducted by Marković et al. (2017b). In this study, peas and oats were tested at five different mixture rates: 100% peas, 100% oats, 25% peas + 75% oats, 50% peas + 50% oats and 75% peas + 25% oats at first pod formation on 2/3 of the pea plants. The highest CP content was observed in the plots of 100% peas, and the lowest CP content was obtained in the plots of 100% oats. Among the mixtures, the highest CP content was found in the 75% pea

mixture. These authors concluded that the CP content increased with increasing pea content in the mixtures due to the high CP content in peas.

In ruminant nutrition, a balance between structural and non – structural carbohydrates and proteins is very important to reduce nutrient losses, such as nitrogen, which is very important in a sustainable production system (Marković et al., 2020a, b). Carbohydrates are important in animal nutrition, because they are the main source of energy and usually make up 70 – 80% of the diet. Cell walls are the main fraction of carbohydrates and play an important role in both plant and animal nutrition. They provide structural support and protection to plants. These functions mean that these components are resistant to degradation, which limits the use of nutrients in animals. In fact, animals do not produce the enzymes needed to digest cell walls, but they have developed beneficial relationships with microorganisms that are able to degrade cell walls.

Table 4. The chemical composition, carbohydrate fractions, and digestibility of herbage of different pea – oat mixtures.

	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	A <sub>5</sub>
DM, g kg <sup>-1</sup>	248.3 <sup>d</sup>	280.0 <sup>a</sup>	271.6 <sup>b</sup>	263.0 <sup>c</sup>	270.3 <sup>b</sup>
CP, g kg <sup>-1</sup> DM	190.3 <sup>a</sup>	114.5 <sup>e</sup>	126.8 <sup>d</sup>	152.8 <sup>c</sup>	167.3 <sup>b</sup>
CHO, g kg <sup>-1</sup> DM	685.3 <sup>d</sup>	753.6 <sup>a</sup>	736.2 <sup>b</sup>	705.6 <sup>c</sup>	697.4 <sup>cd</sup>
NSC, g kg <sup>-1</sup> DM	225.7 <sup>a</sup>	139.5 <sup>d</sup>	179.8 <sup>b</sup>	178.8 <sup>b</sup>	166.6 <sup>c</sup>
Starch, g kg <sup>-1</sup> DM	66.7 <sup>a</sup>	67.7 <sup>a</sup>	65.2 <sup>a</sup>	67.4 <sup>a</sup>	56.5 <sup>b</sup>
NFC, g kg <sup>-1</sup> DM	279.1 <sup>a</sup>	106.5 <sup>e</sup>	148.5 <sup>d</sup>	165.2 <sup>c</sup>	200.4 <sup>b</sup>
aNDF, g kg <sup>-1</sup> DM	435.9 <sup>e</sup>	663.8 <sup>a</sup>	606.2 <sup>b</sup>	564.2 <sup>c</sup>	520.4 <sup>d</sup>
ADF, g kg <sup>-1</sup> DM	352.1 <sup>c</sup>	441.4 <sup>a</sup>	443.5 <sup>a</sup>	415.1 <sup>b</sup>	417.3 <sup>b</sup>
HCL, g kg <sup>-1</sup> DM	76.2 <sup>e</sup>	203.4 <sup>a</sup>	149.1 <sup>b</sup>	136.4 <sup>c</sup>	93.2 <sup>d</sup>
Lignin, g kg <sup>-1</sup> DM	61.6 <sup>b</sup>	81.1 <sup>a</sup>	82.4 <sup>a</sup>	69.8 <sup>b</sup>	82.2 <sup>a</sup>
DMD, g kg <sup>-1</sup> DM	774.0 <sup>a</sup>	579.8 <sup>e</sup>	611.4 <sup>d</sup>	649.8 <sup>c</sup>	691.1 <sup>b</sup>
CA, g kg <sup>-1</sup> CHO	329.4 <sup>a</sup>	185.2 <sup>d</sup>	244.3 <sup>c</sup>	253.4 <sup>b</sup>	238.9 <sup>c</sup>
CB <sub>1</sub> , g kg <sup>-1</sup> CHO	97.4 <sup>a</sup>	89.8 <sup>b</sup>	88.6 <sup>b</sup>	95.5 <sup>a</sup>	81.1 <sup>c</sup>
CB <sub>2</sub> , g kg <sup>-1</sup> CHO	166.8 <sup>a</sup>	77.9 <sup>d</sup>	79.2 <sup>d</sup>	110.7 <sup>c</sup>	147.9 <sup>b</sup>
CB <sub>3</sub> , g kg <sup>-1</sup> CHO	258.4 <sup>e</sup>	452.3 <sup>a</sup>	389.5 <sup>b</sup>	372.9 <sup>c</sup>	334.7 <sup>d</sup>
CC, g kg <sup>-1</sup> CHO	148.0 <sup>b</sup>	194.7 <sup>a</sup>	197.9 <sup>a</sup>	167.4 <sup>b</sup>	197.2 <sup>a</sup>

A<sub>1</sub> – 100% peas + 0% oats; A<sub>2</sub> – 0% peas + 100% oats; A<sub>3</sub> – 25% peas + 75% oats; A<sub>4</sub> – 50% peas + 50% oats; A<sub>5</sub> – 75% peas + 25% oats; DM – dry matter; CP – crude protein; CHO – total carbohydrates; NSC – non-structural carbohydrates; NFC – non-fiber carbohydrates; aNDF – neutral detergent fiber; ADF – acid detergent fiber; HCL – hemicellulose; DMD – dry matter digestibility; CA – instantaneously degradable carbohydrates; CB<sub>1</sub> – starch; CB<sub>2</sub> – intermediately degradable carbohydrates; CB<sub>3</sub> – available cell wall; CC – unavailable cell wall; Different letters in the same row denote significantly different means (P< 0.05); Marković et al. (2017b).

In low production grazing ruminants, plant cell walls represent 70–90% of the total carbohydrates ingested by the animals. Because cell walls are digested slowly and incompletely, the ratio of cell walls in the diets of high producing cows must

be limited, but they still make up 40–60% of the carbohydrates in the diet. The amount of cell walls in the diet of ruminants and the negative impact on the amount of meals ingested and digested by dairy cows show the importance of studying the structure and utilization of cell walls. Discovering the relationship between the chemical nature of cell walls and their utilization will help us to understand and reduce their limiting factor in the diet of dairy cows and achieve that forages are used more efficiently and in greater quantities (Marković et al., 2019a, b).

Kocer and Albayrak (2012) indicated that the content of NDF and ADF increased with decreasing pea content in the mixture. According to the findings of these authors, pure pea crops contained the lowest content of ADF and NDF – 258.1 and 382.7 g kg<sup>-1</sup> DM, respectively, whereas the highest content of ADF and NDF was found in pure oat crops (346.1 and 591.2 g kg<sup>-1</sup> DM, respectively). Aasen et al. (2004) reported that increasing the content of the legume component in mixtures with grasses and cereals contributed to a decrease in the content of NDF and ADF. Van Soest (1996) also pointed out that legumes under similar growing conditions had lower levels of ADF and NDF, whereas cereals contained higher levels. Carr et al. (2004) found lower NDF and ADF levels in the pea – oat mixtures compared to pure oats. These authors also found that growing peas and oats in a bi – crop system contributed to better nutrient digestibility compared to growing oats alone.

Balabanli et al. (2010) studied the content of NDF and ADF in mixtures of common vetch with different cereals – rice, barley, wheat, oats, triticale, and found that the NDF content ranged from 501.1 to 513.9 g kg<sup>-1</sup> DM and the ADF content ranged from 319.2 to 327.9 g kg<sup>-1</sup> DM, respectively. Lithourgidis et al. (2006) found that the NDF content increased as the common vetch seed increased in the mixture. In the pure common vetch crop, the NDF content was 443.1 g kg<sup>-1</sup> DM, while in the pure oat crop the NDF content was 345.3 g kg<sup>-1</sup> DM. The determined value for the NDF content in the mixture with a ratio of common vetch to oats of 55:45 was 367.7 g kg<sup>-1</sup> DM, and the NDF content was 401.7 g kg<sup>-1</sup> DM when the ratio of common vetch to oats was 65:35. These authors found that pure common vetch had the highest NDF content, which is in contrast to the results obtained by Caballero et al. (1995) and Assefa and Ledin (2001). The reason for these results could be that different legume cultivars were used or the legumes were harvested at different stages of development. In the mixture where the ratio of common vetch to oats was 55:45, these authors found 387.0 g kg<sup>-1</sup> DM of ADF and 55.5 g kg<sup>-1</sup> DM of lignin, while in the mixture where the ratio of common vetch to oats was 65:35, the ADF content was 351.4 g kg<sup>-1</sup> DM, while the lignin content was 55.8 g kg<sup>-1</sup> DM. They found that increasing the proportion of legumes in the mixture increased the lignin content, which can be explained by the fact that the cell walls of cereals contain less lignin compared to the cell walls of legumes (Carpita and McCann, 2000). The same authors found in these studies that the DMD of the pure common

vetch crop was  $604.0 \text{ g kg}^{-1}$  DM, the DMD of the pure oat crop was  $602.7 \text{ g kg}^{-1}$  DM, while in a mixture where the ratio of common vetch to oats was 55:45, the DMD was  $587.5 \text{ g kg}^{-1}$  DM, and in the mixture where this ratio was 65:35, the DMD was  $615.2 \text{ g kg}^{-1}$  DM. Caballero et al. (1995) pointed out that an adequate contribution of common vetch to forage quality can only be achieved if the proportion of oat seeds in the mixture is 10–20%.

Assefa and Ledin (2001) studied the NDF content in different oat varieties, and found that the NDF content ranged from  $628$  to  $661 \text{ g kg}^{-1}$  DM, while the IVOMD (*In vitro* Organic Mater Digestibility) ranged from  $540$  to  $589 \text{ g kg}^{-1}$  DM. The content of NDF in the leaf was  $622 \text{ g kg}^{-1}$ , and in the stem  $742 \text{ g kg}^{-1}$ , while the DMD of the leaf was  $613 \text{ g kg}^{-1}$  DM and the DMD of the stem was  $372 \text{ g kg}^{-1}$  DM. These authors found that legumes were characterized by better nutritional value, but lower DM yields per hectare. Nsahlai and Umunna (1996) indicated that the content of CP and NDF in a similar experiment conducted with mixtures of oats and common vetch had a direct influence on the DM intake and DMD of forages.

Legumes contribute to higher levels of nitrogen and other minerals in the rumen, which increases the activity of rumen microorganisms, and thus improves the conditions for better digestion and passage rate of food through the digestive tract (Bonsi et al., 1994). Feeding animals mixtures of legumes and oats will therefore improve the DM intake, passage rate and digestion of food. These observations were confirmed in the study of Abule et al. (1996), who found a better DM intake of a barley – legume mixture. On the other hand, there are a number of factors that influence the fiber requirements of dairy cows, including DM intake, the amount and type of non – structural and structural carbohydrates in the diet, particle size and nutrient processing, and the rate and extent of fiber fermentation processes. A better knowledge of these factors is necessary to achieve maximum energy intake in early lactation. NRC (2001) recommends that the NDF and ADF content of the meal should be 25–28% and 19–21%, respectively, and that 75% of the NDF should come from forages.

Fibers consist of an indigestible fraction and one or more digestible fractions, and each of these fractions has a special rate of degradation. The process of fiber digestion consists of hydrolysis of polysaccharides and conversion of monosaccharides into volatile fatty acids, gases produced during fermentation, and heat (Tamminga, 1993). The degree of hydrolysis depends on how much the enzymes that break down the complex formed between lignin and carbohydrates can penetrate it (Chesson, 1988). The degree of digestion depends on the size of the indigestible fraction and the relationship between the degree of degradability and the passage of the fibers through the rumen into the digestive system. As the digestibility of fiber derived from forages varies depending on the species and category of animals, these variations may be due to differences in the composition of forages, depending on the time of harvest. The indigestible fraction of the NDF

is the main factor influencing the utilization of fibrous carbohydrates and can make up more than half of the total NDF in the rumen. Therefore, there must be a balance between the total fibers and the indigestible fiber fractions (Varga et al., 1998).

The results of the study conducted by Marković et al. (2018b) are presented in Table 5. The average values for the NDF content of common vetch at different growth stages showed that the NDF content increased from flowering (568.7 g kg<sup>-1</sup> DM) to the stage of first pod formation (583.6 g kg<sup>-1</sup> DM), but did not differ significantly.

Table 5. The forage quality of intercropped common vetch (*Vicia sativa* L.) and oats (*Avena sativa* L.).

Factors		DMY t ha <sup>-1</sup>	CP g kg <sup>-1</sup> DM	CPY kg ha <sup>-1</sup>	ADF g kg <sup>-1</sup> DM	NDF g kg <sup>-1</sup> DM	Lignin g kg <sup>-1</sup> DM	DMD g kg <sup>-1</sup> DM
A	B							
a <sub>1</sub>	b <sub>1</sub>	7.00 <sup>h</sup>	183.7 <sup>c</sup>	1285.9 <sup>j</sup>	440.4 <sup>b</sup>	509.2 <sup>g</sup>	88.0 <sup>d</sup>	649.9 <sup>b</sup>
	b <sub>2</sub>	13.82 <sup>b</sup>	99.5 <sup>i</sup>	1375.1 <sup>h</sup>	404.5 <sup>e</sup>	627.0 <sup>b</sup>	61.3 <sup>b</sup>	589.7 <sup>d</sup>
	b <sub>3</sub>	13.13 <sup>c</sup>	125.3 <sup>g</sup>	1645.2 <sup>ab</sup>	432.9 <sup>c</sup>	575.8 <sup>d</sup>	75.8 <sup>g</sup>	619.2 <sup>c</sup>
	b <sub>4</sub>	11.06 <sup>ef</sup>	147.2 <sup>e</sup>	1628.0 <sup>b</sup>	415.0 <sup>d</sup>	562.2 <sup>d</sup>	74.8 <sup>g</sup>	620.9 <sup>c</sup>
	b <sub>5</sub>	9.56 <sup>g</sup>	155.3 <sup>d</sup>	1484.7 <sup>f</sup>	441.0 <sup>b</sup>	569.2 <sup>d</sup>	86.5 <sup>e</sup>	628.6 <sup>c</sup>
Average A <sub>1</sub>		10.91	142.2	1551.4	426.8	568.7	77.3	621.6
a <sub>2</sub>	b <sub>1</sub>	6.21 <sup>i</sup>	213.4 <sup>a</sup>	1325.2 <sup>i</sup>	434.0 <sup>c</sup>	527.7 <sup>f</sup>	96.9 <sup>b</sup>	656.7 <sup>a</sup>
	b <sub>2</sub>	13.97 <sup>b</sup>	114.5 <sup>b</sup>	1599.5 <sup>c</sup>	441.4 <sup>b</sup>	663.8 <sup>a</sup>	81.1 <sup>f</sup>	579.8 <sup>d</sup>
	b <sub>3</sub>	12.81 <sup>cd</sup>	121.6 <sup>g</sup>	1557.7 <sup>d</sup>	448.9 <sup>a</sup>	597.0 <sup>c</sup>	91.1 <sup>c</sup>	567.3 <sup>e</sup>
	b <sub>4</sub>	12.12 <sup>d</sup>	124.3 <sup>f</sup>	1627.7 <sup>b</sup>	413.8 <sup>d</sup>	569.9 <sup>d</sup>	85.2 <sup>e</sup>	574.7 <sup>e</sup>
	b <sub>5</sub>	10.60 <sup>f</sup>	157.6 <sup>d</sup>	1670.5 <sup>a</sup>	419.5 <sup>d</sup>	559.5 <sup>e</sup>	88.3 <sup>d</sup>	566.5 <sup>e</sup>
Average A <sub>2</sub>		11.14	148.3	1652.0	431.5	583.6	88.5	589.0
a <sub>3</sub>	b <sub>1</sub>	5.81 <sup>j</sup>	200.1 <sup>b</sup>	1162.5 <sup>k</sup>	428.0 <sup>c</sup>	520.1 <sup>f</sup>	100.8 <sup>a</sup>	638.6 <sup>b</sup>
	b <sub>2</sub>	14.68 <sup>a</sup>	97.0 <sup>i</sup>	1423.9 <sup>g</sup>	396.6 <sup>f</sup>	578.8 <sup>d</sup>	89.9 <sup>d</sup>	536.1 <sup>g</sup>
	b <sub>3</sub>	11.50 <sup>e</sup>	130.9 <sup>f</sup>	1505.3 <sup>e</sup>	397.1 <sup>f</sup>	548.0 <sup>e</sup>	90.9 <sup>c</sup>	565.4 <sup>e</sup>
	b <sub>4</sub>	9.86 <sup>g</sup>	141.6 <sup>e</sup>	1396.2 <sup>h</sup>	399.2 <sup>f</sup>	551.5 <sup>e</sup>	95.1 <sup>b</sup>	552.2 <sup>f</sup>
	b <sub>5</sub>	9.06 <sup>g</sup>	145.5 <sup>e</sup>	1318.2 <sup>i</sup>	401.2 <sup>e</sup>	551.9 <sup>e</sup>	93.4 <sup>c</sup>	574.4 <sup>e</sup>
Average A <sub>3</sub>		10.18	143.0	1455.7	404.4	550.1	94.0	573.3
Average B <sub>1</sub>		6.34	199.1	1262.3	454.2	519.0	95.2	648.3
Average B <sub>2</sub>		14.15	103.7	1467.4	414.2	623.2	77.4	548.5
Average B <sub>3</sub>		12.48	125.9	1571.2	426.3	573.6	85.9	584.0
Average B <sub>4</sub>		11.01	141.0	1552.4	409.4	561.2	85.0	582.6
Average B <sub>5</sub>		9.74	152.8	1488.3	420.6	560.2	89.4	589.8

A – growth stage; B – mixture rate; DMY – dry matter yield; CP – crude protein; CPY – crude protein yield; ADF – acid detergent fiber; NDF – neutral detergent fiber; DMD – dry matter digestibility; a<sub>1</sub> – vetch flowering initiation – 10% of flowering; a<sub>2</sub> – forming the first pods on 2/3 of vetch plants and a<sub>3</sub> – forming green seeds in 2/3 of pods; b<sub>1</sub> – 100% vetches + 0% oats; b<sub>2</sub> – 0% vetches + 100% oats; b<sub>3</sub> – 40% vetches + 60% oats; b<sub>4</sub> – 50% vetches + 50% oats and b<sub>5</sub> – 60% vetches + 40% oats; Different letters in the same column denote significantly different means (P< 0.05), Marković et al. (2018b).

As growth and development continued, the NDF content decreased by 5.75% (Table 5). The lowest NDF content was found in pure vetches (519.0 g kg<sup>-1</sup> DM), while the highest NDF content was found in pure oats (623.2 g kg<sup>-1</sup> DM). The highest lignin content was found in pure common vetch (95.2 g kg<sup>-1</sup> DM). According to the fact that common vetch contained a significantly higher lignin content, the mixture with the highest proportion of common vetch contained the highest lignin content (89.4 g kg<sup>-1</sup> DM). The dry matter digestibility of the vetch – oat mixtures studied decreased by 8.42% with growth and development. Pure common vetch was 14% more digestible than pure oats, whereas the common vetch seeding rate did not contribute to the better digestibility of vetch – oat mixtures.

Early lactating cows fed diets containing more digestible fibers consumed 1.18 kg more DM and produced 1.23 kg more milk per day than cows fed diets containing less digestible fibers. The NDF concentration in the ration is negatively correlated with the DM intake because the fibers ferment more slowly and remain longer in the rumen compared to other nutrient components (Varga et al., 1998). Allen and Oba (1986) reported that a 1– unit increase in digestion led to a higher milk production of 0.23 kg per day.

Aasen et al. (2004) reported that increasing the seeding rate of leguminous components in the mixtures with grasses and cereals contributed to the reduction of NDF and ADF content. Lithourgidis et al. (2006) also found that NDF content increased with an increase in the proportion of germinated common vetch in the mixtures. Carpita and McCann (2000) found that the lignin content increased with increasing the seeding rate of common vetch in the mixtures, which could be explained by the fact that the cell wall of cereals contains less lignin compared to the cell wall of legumes.

### Digestibility of legume – cereal intercrops

The digestibility of organic matter is one of the most important quality parameters of forage plants (Buxton and Redfearn, 1996). Nutritive value changes during plant growth and development, primarily due to a decrease in the leaf to stem ratio (Lloveras, 2001; Guines et al., 2003). In the leguminous stem, the amount of fiber increases over time, and its fermentation in the rumen is partial and slow, directly limiting the available energy, while the digestibility of leaves is relatively constant and high (Buxton and Redfearn, 1996; Guines et al., 2003). Lignified cell walls have a higher lignin content, which is responsible for the lower digestibility. On the other hand, lignified tissues are necessary to ensure the mechanical resistance of the plants, which practically limits the possibilities to increase the quality of forage plants (Buxton and Redfearn, 1996).

The ability to provide adequate energy levels to high – producing dairy cows is dependent on how precisely the feed composition can be determined. Rumen microorganisms use carbohydrates as energy sources to synthesize microbial protein for their growth, which is important for normal rumen function. Carbohydrates are the main source of energy for animals, necessary for maintenance and milk production. They are also precursors in the synthesis of lactose, fat and protein. Formulation of rations based on NDF content is recommended because of the positive correlation between NDF concentration and rumen capacity and the negative correlation between NDF and energy values of nutrients (Mertens, 1994).

Marković et al. (2018c) found the highest DMD in the pure pea crop (744.9 g kg<sup>-1</sup> DM) and the lowest in the pure oat crop (562.9 g kg<sup>-1</sup> DM). The results of this study showed that DMD in mixtures decreased with increasing the pea seeding rate from 25:75 to 50:50 in the pea – oat mixture. Marković et al. (2020b) also indicated that the DMD of pea and oat silages was significantly influenced by the structure of the mixtures and the stage of plant development and that DMD was greatly influenced by ADF and lignin content. The DMD of pea – oat mixtures decreased by 6.4% from the first to the second stage of plant development and by 9.25% up to the third stage of development. The decrease in dry matter digestibility was due to the increase in the amount of structural carbohydrates – NDF and ADF – and intensive lignification of the cell wall as the plant grew and developed. The DMD of the pure pea crop was 32.7% higher than the DMD of the pure oat crop. Due to the higher digestibility of the pea crop, higher digestibility was observed in the mixtures with a higher proportion of peas in the mixtures.

The results obtained in the study conducted by Marković et al. (2018b) showed that the DMD of the examined common vetch – oat mixtures decreased with plant growth and development from 621.6 to 573.3 g kg<sup>-1</sup> DM. The highest DMD was observed in the first stage of development and was 5.3% higher than in the second stage of development. The lowest DMD was observed in the third stage of development and was 2.7% lower than in the second stage of development. The pure common vetch cultivation was characterized by a 14% higher DMD compared to the pure oat cultivation. In the common vetch – oat mixtures studied, the proportion of common vetch in the mixtures did not contribute significantly to better digestibility.

## Conclusion

These results illustrate that CP and cell wall components as well as dry matter digestibility of pea – oat and common vetch – oat mixtures were affected by the growth stage and seeding rate of pea, common vetch and oats in the mixtures. The data obtained from these studies show that pea – oat and common vetch – oat

mixtures can be successfully grown for forage production. Growing peas and oats and common vetch and oats in mixtures proved to be beneficial for increasing forage quality. In general, pure oats contained a significantly higher content of cell wall components than pure pea and common vetch crops. The higher proportion of legumes in the mixtures influenced the better nutritive value of feeds investigated. The structure of the mixtures also significantly affected the lignin content. It was also determined that all pea – oat and common vetch – oat mixtures studied can provide high quality forage suitable for high animal production. We recommend harvesting vetch – oat mixtures when the first pods have formed on 2/3 of the vetch plants. Regarding the most favorable mixture depending on the seeding rate of common vetch and oats in the mixtures, we recommend a 1:1 common vetch – oat mixture. On the other hand, 25:50 and 50:50 pea – oat mixtures could be recommended for ruminant feeding.

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## HRANLJIVA VREDNOST ZDRUŽENIH USEVA GRAŠKA I OVSA I GRAHORICE I OVSA

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### R e z i m e

Grašak i grahorica predstavljaju značajne biljne vrste za proizvodnju kabaste stočne hrane, pri čemu se mogu koristiti kao zelena hrana, zatim u vidu sena ili silaže. Zbog svojih specifičnosti (sklonosti ka poleganju i visokog puferskog kapaciteta) najčešće se gaje u smeši sa strnim žitima, kao potpornim usevima, kako bi se na taj način sprečilo ili umanjilo poleganje, smanjio puferski kapacitet, a ujedno dobila biomasa pogodna za proces siliranja. U čistim usevima grašak se kao zelena hrana ili seno najčešće koristi u fazi formiranja prvih mahuna, dok se najbolji kvalitet suve materije kod grahorice postiže njenim iskorišćavanjem u fazi punog cvetanja ili početka obrazovanja prvih mahuna kada se dobija i do 25% sirovih proteina u biomasi čiste kulture. U ovom radu je prikazana hranljiva vrednost čistih useva graška, grahorice i ovsa, i njihovih smeša u zavisnosti od strukture smeše i faze iskorišćavanja. Posebna pažnja je posvećena sadržaju komponenata čelijskih zidova i svarljivosti suve materije ovih hraniva. Zbog svega navedenog je od posebne važnosti odabrati pravi momenat kosidbe kako bi se na taj način mogao uskladiti prinos i hranljiva vrednost dobijenih hraniva.

**Ključne reči:** združeni usev graška i ovsa, združeni usev grahorice i ovsa, hranljiva vrednost.

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## SUSTAINABILITY AND PERSPECTIVES OF THE NORTH MACEDONIAN DAIRY INDUSTRY

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**Abstract:** The subject of this review is the dairy industry in the Republic of North Macedonia. This industry can bring many economic and social benefits to society and the economy. The paper provides a description and analysis of the current situation within the dairy industry in our country. Milk production rates in our country are analyzed, with a comparative approach to production rates at global and European levels. In the Republic of North Macedonia, milk production is characterized by many traditional small farmers (80% of the total) with 1–3 cows and low annual milk productivity (2–3 thousand liters per cow). The number of large specialized farms with more than 15 cows (about 3% of the total) with annual milk production of about 5 thousand liters per cow is minimal. Only 1% of all farms have more than 50 cows. The Republic of North Macedonia is an absolute importer of milk and its products. The quantity and monetary value of imported milk and its products are many times higher than the quantity and value of exported milk. Today, the dairy industry faces various challenges in the three main pillars of sustainability: economic, social and environmental including fluctuating market prices, high labor costs, and demographic characteristics of the population in terms of age. In conclusion, for the dairy industry to be sustainable, all activities should be carried out in partnership with the industry (along the value chain), policymakers and a wide range of stakeholders, including consumers.

**Key words:** dairy industry, financial support, milk quality, dairy product standards.

### Introduction

The dairy industry is a branch of the food industry that deals with the production and processing of milk and its products. Global milk production reached 906 million tons during 2020, registering a 2% increase compared to the 2019 data (FAO, 2021). As a branch of economic activity, it is based on livestock production which is also presented as the primary source of necessary raw

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materials. Dairy industry refers to the industry or business of production and processing of animal milk, in our case cow's, sheep's and goat's milk and in some cases other animals for daily consumption by humans. Augustin et al. (2013) and Von Keyserlingk et al. (2013) have highlighted concerns in the literature about the sustainability of the dairy industry in the face of climate change, globalization and a lack of multidisciplinary research initiatives. Developed countries, which represent the countries with the largest volume of fresh milk production, account for the largest number of studies. However, there are an increasing number of publications in some developing countries such as India and Turkey, possibly due to the presence of this industry in these countries (Feil et al., 2020).

Generally, the dairy farm is the one that produces milk, while the dairy factory is the one that processes it and converts it into a variety of dairy products. These enterprises, like farms or factories, are part of the global dairy industry of the food industry. Nowadays, more than 6 billion people worldwide consume milk, butter, cheese, yoghurt and other dairy products. Per capita milk consumption has almost doubled in developing countries since the early 1960s. Given that meat and egg consumption has tripled and quintupled, respectively, milk consumption has quiet grown (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>). Dairy products vary significantly from country to country in the same region, depending on cultural and social dietary habits, technology, and market demand. Demand for dairy products is increasing in developing countries with population growth, urbanization, rising incomes, and changing diets. The gap with many developing countries is narrowing, although the per capita consumption of dairy products is higher in developed countries. The increasing demand for dairy products provides a very good opportunity for producers and other players in the dairy chain to improve their standard of living through increased production. The most consumed dairy product in developing countries is fluid milk, but dairy products are also playing an increasingly important role in many countries (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>). As a fundamental part of the agricultural complex in the Republic of North Macedonia, the dairy industry occupies an important place in developing the country's overall economy and the international foreign exchange market. Despite the influence of imports of dairy products, there are opportunities for the progress of the Macedonian dairy sector. Significant improvement in the dairy sector is ensured by the modernization process of primary production and advanced training of dairy processors (Bojkovska and Petkovska, 2016). Codex Alimentarius defines a dairy product as a product obtained from the processing of milk and containing food additives and other ingredients functionally necessary for processing (<https://www.fao.org/fao-who-codexalimentarius/en/>).

### The dairy industry in North Macedonia

The agricultural and agribusiness sectors are the most significant economic sectors of North Macedonia. Nearly 433,500 people, out of a population of 2,083,173, derive their livelihood or part of their income from agricultural services. The excellent natural conditions and the present heritage, enriched with new advanced skills offer diverse opportunities in agriculture, from pastures in the high mountains with an altitude of over 2000 meters to a rich Mediterranean climate with water valleys. One of the rapidly developing sectors in North Macedonia is the agribusiness sector. In view of the large European markets, the processing of high-quality agricultural products is one of the most important business areas. Organic production in North Macedonia is growing very fast. In the last four years, the area approved for organic production has increased. According to statistics, there are more than 800 organic farmers in our country, focused on the production of meat, milk and dairy products, honey, cereals, industrial oil crops, wine, fruits and vegetables (<https://investnorthmacedonia.gov.mk/export-agrobusiness-and-food-processing/>).

About half of the territory of the Republic of North Macedonia is agricultural, of which 44% is arable land and 56% is pasture land. North Macedonia has a significant raw material base from primary agriculture, which is the basis for the development of the agricultural and processing sector (<https://www.mchamber.mk/Default.aspx?mId=19&id=29&lng=1>).

The agri-food sector is one of the most important sectors of the national economy, or the third most important sector after industry and services, accounting for 18% of the gross domestic product (GDP), with agriculture accounting for about 12% and the food industry accounting for 6% of GDP (<https://www.mchamber.mk/Default.aspx?mId=19&id=29&lng=1>). The dairy industry is the most significant agricultural industry in North Macedonia. White cheese is a brined cheese type with salty taste and firm texture. It is an indigenous dairy product with a long history and is the most consumed cheese in the country (Sulejmani et al., 2021). It is similar to the various country cheeses on the Balkan Peninsula, included in the category of indigenous dairy products depending on the area, production technology, name and quality (Mateva et al., 2019; Sulejmani et al., 2014). The production of brined cheeses has been practiced for centuries, with differences in technological processes, climatic conditions, and cultural habits. However, industrial standardized production has increased in the last decade of the century (Huppertz et al., 2006; Sulejmani, 2014). Little is known about Kashkaval cheese produced in Macedonia in terms of production methods and composition (Mijačević and Bulajić, 2004; Santa and Srbinovska, 2014). An exceptional contribution is made by the diversity of geographical conditions, which contributes to the diversity of types and recipes of dairy products. The milk and dairy industry

is one of the most important segments of the agro-industrial system, and its products belong to the group of food products that make up a significant part of the total food consumption of the average family in our country (<https://www.mchamber.mk/Default.aspx?mId=19&id=29&lng=1>).

To ensure the best quality of milk and its products, it is necessary to ensure high quality milk production. This is essential in view of the country's aspiration to accelerate its membership in the European Union (EU) with its high standards and rules in the dairy production chain. The European Union is a substantial producer of milk and dairy products, integrated into the standard market organization. Milk production takes place in all EU Member States and represents a significant part of the value of EU agricultural inputs. The dairy industry accounts for more than 12% of total EU agricultural production, making it the second-largest agricultural sector after vegetables and horticulture (Bas – Defossez et al., 2019a).

### Milk production

The production of milk and dairy products in the Republic of North Macedonia has a long tradition, to which the diversity of geographical conditions makes a unique contribution, which in turn contributes to the diversity of types and recipes of dairy products. On the Macedonian territory, the administrative ban on goat breeding in 1947 had disastrous consequences; however, goat production increased again in 1989 (Dzabirski and Andonov, 1997). The milk and dairy industry is one of the most critical segments of the agro-industrial system, and its products belong to the group of food products that constitute a significant part of the total food consumption of an average family in our country.

Table 1. Total milk production over the last decade.

Year	Total milk production in'000 liters	Cow's milk		Sheep's milk	
		Total in'000 liters	Average per cow	Total in'000 liters	Average per sheep
2010	394.33	347.10	2.787	32.15	60
2011	417.23	376.29	2.866	25.38	51
2012	403.23	349.76	2.928	38.61	79
2013	429.40	380.73	3.009	34.27	70
2014	436.25	387.00	3.053	35.66	74
2015	417.38	361.07	2.828	40.74	77
2016	463.44	403.04	3.046	41.06	77
2017	448.75	394.14	2.867	35.36	69
2018	463.63	404.23	3.077	36.55	68
2019	442.85	390.90	3.438	35.08	68

The export strategy of North Macedonia for milk and products should expand the volume and improve the export configuration to increase the volume of international exchange and gradually reduce the deficit in the current account (Bojkovska, 2012). The situation of the dairy industry in North Macedonia is presented in Table 1 (SSO, 2020). The fluctuations in terms of total milk production from Table 1 show that the highest level of milk production was observed in 2018, which decreased in subsequent years with an increasing trend. High and low amounts are observed in the yield of cow's milk produced, with the production level being higher in the previous year. In terms of the amount of cow's milk produced per capita, Table 2 shows the overall stability of the amount of milk with a steady upward trend. The most considerable amount of milk per capita was observed in 2020, with a value of 3,648 liters, which is significantly lower than the European average (Table 2). Cow's milk production reaches a value of 405 thousand liters and marks an increase of 3.7% compared to the 2019 data (SSO, 2020). According to the most recent data, the amount of milk significantly decreased in 2021, reaching a total of 389,210 liters ([https://ec.europa.eu/eurostat/databrowser/view/apro\\_mk\\_farm/default/table?lang=en](https://ec.europa.eu/eurostat/databrowser/view/apro_mk_farm/default/table?lang=en)).

Regarding the milk production from sheep and goats in our country, Table 2 shows a downward trend in the amount of milk produced, with the year of 2020 being exactly the lowest level of production of this milk over the last decade. There is a constant decrease in the amount of milk produced (per capita), with just the last year showing the lowest level of milk production from sheep and goats per capita.

While sheep milk production decreased by 23.3% compared to the previous year (2019), goat milk production decreased by 7.7% compared to 2019 (SSO, 2020).

Table 2. Cow, sheep and goat milk production in 2020.

	Number	Average (liters)	Milk production, in 000 liters	Productivity index (2020/2019)
Cow				
Business entities	3.663	6.426	23.539	123.5
Agricultural holdings	107.420	3.553	381.678	102.6
Total	111.083	3.648	405.217	103.7
Sheep				
Business entities	4.217	45	189	31.6
Agricultural holdings	446.894	60	26.735	77.5
Total	451.111	60	26.924	76.7
Goat				
Business entities	758	44	33	33.0
Agricultural holdings	69.374	224	15.534	92.7
Total	70.132	222	15.567	92.3

Cow's milk is the source of most dairy products in terms of production. With about 56.5 million cows, India tops the list of countries with the most dairy cows, while the European Union is the largest producer of cow's milk in the world, followed by the United States. However, China has tripled its milk production since 2000 (<https://www.statista.com/topics/4649/dairy-industry/#dossierKeyfigures>).

One-fifth, or more precisely 21.5%, of the total amount of cow's milk produced at the European level is precisely collected from farms in Germany, and farms process an approximate percentage, or 22.4%, of the total European milk in Germany. Meanwhile, Germany and France, the Netherlands and Poland supplied about two-thirds or 65% of the total amount of raw cow's milk in 2020 ([https://ec.europa.eu/eurostat/statisticsexplained/index.php?title=Milk\\_and\\_milk\\_product\\_statistics](https://ec.europa.eu/eurostat/statisticsexplained/index.php?title=Milk_and_milk_product_statistics)).

Dairy production in our country is mainly characterized by low productivity and an incomplete identification system, which makes it difficult to monitor and control diseases in domestic animals and significantly affects the quality and safety of milk (<https://www.mchamber.mk/Default.aspx?mId=19&id=29&lng=1>).

Primary production also remains a fundamental problem: providing cheap food, more modern breeding conditions, improving the breed composition of livestock, improving the microbiological quality of milk and others. With this quality, the milk arrives at the dairy, where the problems are further aggravated. The particular nature of milk production, distribution and transportation makes the concept of milk control difficult to implement, and the nature of the control itself is quite expensive. Many dairies do not meet basic hygiene and sanitary requirements, and it is not uncommon to have unregistered processing plants. Existing standards and procedures for proper technological operations are not followed, and there is a lack of proper control of incoming material and control at the factory in terms of product quality and safety. The need to intervene in primary production stems from the introduction of standards that establish basic requirements for livestock production (good agricultural practices) and general hygiene rules in primary production (<https://www.mchamber.mk/Default.aspx?mId=19&id=29&lng=1>). It allows for strictly controlled production of raw milk, which must be purchased by processing plants, based on mutual agreements with predetermined conditions and certain rights and obligations of both partners. The overall milk production shows a decreasing trend. The milk produced is used fresh or processed into yoghurt, and cheese. By comparison, there are about 700,000 dairy farms in the European Union. There are about 12,000 production and processing stations. This industry creates about 300,000 jobs directly related to milk processing. The dairy industry generates about 10 billion euros in the EU trade balance. In addition, 10 of the world's top 20 dairy companies are from the European Union. With more than 300

registered cheeses and other dairy products, the dairy industry also contributes to the EU's cultural heritage (EDA, 2020).

About 20 million cows with an average of 7,300 liters of milk were in the EU in 2020 ([https://ec.europa.eu/info/food-farming-fisheries/animals-and-animal-products/animal-products/milk-and-dairy-products\\_en](https://ec.europa.eu/info/food-farming-fisheries/animals-and-animal-products/animal-products/milk-and-dairy-products_en)). There are many differences in the quantity, form and size of farms involved in the production and processing of milk and its products. However, despite many challenges, it can be observed that the less developed producers of milk and its products are rapidly trying to converge with the producers who apply a more structured and modern approach to production when they are even more visible. Milk production produced by smallholder farmers contributes to the livelihood of families in most developing countries, as is the case with the processing of milk and its products in our country (Al Sidawi et al., 2021). Developing countries have increased their milk production worldwide in recent decades, more due to an increase in the number of animals than to an increase in productivity. This increase is mainly due to an increase in the number of productive animals rather than an increase in productivity per animal (FAO, 2009; Thornton, 2010). In many developing countries, milk productivity is constrained by inferior food sources, diseases, limited access to markets and services, and the low genetic potential of dairy animals for milk production, including our country. In some developed countries, milk production has a long tradition and its products play an important role in nutrition. Last year, raw milk production on farms in the EU amounted to about 160.1 million tons, an increase of 1.9 million tons compared to the previous year. This figure amounts to 154.4 million tons of cow's milk, 3.0 million tons of sheep's milk, 2.5 million tons of goat's milk, and 0.3 million tons of buffalo's milk. Most of the milk is used as fresh milk for beverages, while the rest is used for the production of cheese, milk powder, butter and ash. Last year, the amount of milk per capita increased, reaching 7,509 liters of cow's milk per capita. At the national level, the largest increase in the quantity of milk per capita is observed in Estonia, with about 10,063 liters and in Denmark with 10,028 liters per capita, while the lowest quantity of milk is recorded in Bulgaria (3,654 liters) and Romania (3,228 liters). However, Germany and the Netherlands are well above the European average ([https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Milk\\_and\\_milk\\_product\\_statistics](https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Milk_and_milk_product_statistics)).

## Market and trade

Trade in milk and dairy products improves revenues, creates business in production, distribution, marketing, and food security. Globalization has resulted in milk and its products being consumed in every corner of the world. Each year, large volumes of milk, cheese and other milk products are marketed among

different countries. In 2020, milk and dairy products worth over \$55.75 billion were exported, marking a significant increase of nearly \$39 billion compared to 2015. With countries such as France, Ireland and Germany, the European Union controls 38% of the export market for milk and dairy products. Germany alone exports cheese worth about \$4.6 billion annually.

In recent years, there have been many creative and innovative efforts related to the processing, fermentation and consumption of milk and dairy products. In 2019, the value of the global market of milk and its products was estimated at \$720 billion, which is expected to reach the value of \$1,032 billion in 2024 (<https://www.statista.com/topics/4649/dairy-industry/#dossierKeyfigures>).

In developing countries, most milk is traded through informal channels, i.e., without regulation or licensing (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>). The situation is similar, especially in rural areas where consumers prefer to process milk by boiling. As a result, the variance between producer and consumer prices in the informal market is generally negligible.

The most controlled agricultural market is the dairy market. In developing countries, strategies usually focus on minimizing dairy imports and promoting national production, increasing domestic farm income, and minimizing import costs. However, it is likely that milk production will not be sufficient in the future and therefore more and more dairy products will have to be imported.

Because many dairy products are highly perishable, the percentage of dairy products traded internationally is low, usually 8% (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>).

Table 3. The volume of purchases of milk and its products during the last decade (expressed in tons).

	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Fresh cow's milk, in '000 liters	83.997	106.466	105.711	113.760	135.996	137.467	148.381	140.055	153.068	148.786
Fresh sheep's milk, in '000 liters	2.350	5.376	3.617	6.752	8.941	6.955	8.297	5.924	8.031	6.096
Käckavall (trappist)	46	122	6	7	10	103	470	351	176	124
Other cheeses	2.017	302	786	824	1.216	635	1.397	711	401	435
Other dairy products	91	615	248	83	520	265	628	436	113	63

Butter, cheese and milk powder are the most traded dairy products on the world market. In our country, according to the State Statistical Office, the total amount of purchases of milk and dairy products during the last decade is shown in

Tables 3, 4, and 5 (SSO, 2015–2020). The total value of purchases of milk and dairy products over the last decade is shown in Table 4.

Table 4. The total value of purchases of milk and dairy products during the last decade (expressed in '000 denars).

	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Milk and dairy products	1.565.789	2.216.384	2.369.203	2.761.345	3.321.302	3.035.684	3.296.076	2.945.747	3.387.310	3.244.401
Fresh cow's milk	1.426.372	1.930.492	2.076.314	2.362.653	2.775.787	2.648.019	2.652.916	2.489.998	2.999.419	2.936.380
Fresh sheep's milk	68.343	152.578	108.310	210.391	268.007	208.829	262.936	181.635	254.887	193.628
Kaçkaval (trapist)	18.010	41.447	2.346	3.138	2.802	29.690	124.800	103.182	48.767	33.265
Other cheeses	45.123	55.038	167.840	177.573	251.167	133.336	224.846	145.558	76.969	77.791
Other dairy products	7.941	36.829	14.393	7.591	23.538	15.810	30.577	25.374	7.270	3.336

The total amount of milk and its products purchased by each producer over the last decade is shown in Table 5. The increase of about 100% in the total volume and quantities of milk purchased by 2018 shows the intensification of the production system in this sector in the last decade, which could be attributed to the use of improved genetic material (Koutouzidou et al., 2022).

Table 5. Total amount (expressed in tons) and value (expressed in '000 denars) of purchases of milk and dairy products.

	2010	2011	2012	2013	2014
Fresh cow's milk, in '000 liters	70.173	90.829	88.793	101.507	113.409
Fresh sheep's milk, in '000 liters	2.122	5.287	3.173	6.407	8.713
Kaçkaval (trapist)	5	1	-	-	-
Other cheeses	3	2	47	63	134
	2015	2016	2017	2018	2019
Other dairy products	3	22	3	1	4
Fresh cow's milk, in '000 liters	118.240	127.093	116.465	132.604	128.993
Fresh sheep's milk, in '000 liters	6.749	7.575	5.538	7.261	5.705
Kaçkaval (trapist)	22	50	8	13	12
Other cheeses	267	291	32	39	20
Other dairy products	-	-	56	19	-
Value (expressed in '000 denars)					

Continuation Table 5. Total amount (expressed in tons) and value (expressed in '000 denars) of purchases of milk and dairy products.

	2010	2011	2012	2013	2014
Milk and dairy products	1.269.141	1.788.073	1.811.636	2.300.008	2.583.093
Fresh cow's milk	1.203.563	1.636.314	1.711.802	2.088.628	2.285.489
Fresh sheep's milk	62.618	149.822	95.115	200.311	260.460
Kaçkavall (trapist)	2.160	197	-	-	-
Other cheeses	500	287	4.454	10.986	36.957
Other dairy products	300	1.454	265	82	186
	2015	2016	2017	2018	2019
Milk and dairy products	2.513.069	2.542.706	2.190.149	2.772.714	2.686.492
Fresh cow's milk	2.252.878	2.230.780	2.013.829	2.532.382	2.499.999
Fresh sheep's milk	200.335	240.103	167.113	227.492	179.724
Kaçkavall (trapist)	6.443	15.094	2.205	3.497	3.339
Other cheeses	53.414	56.728	5.891	7.295	3.429
Other dairy products	-	-	1.112	2.047	-

This trend indicates that dairy farms have increased the use of capital in modern buildings and equipment. Strengthening production structure through increasing herd size and optimal feeding as a persistent strategy for modern dairy farms was also confirmed by Wilson (2011), Mitsopoulos et al. (2011) and Poczta et al. (2020).

The trade balance of the dairy industry and milk and dairy products is shown in Table 6. As stated by the available data, we assume that the quantity and monetary value of milk and its imported products are many times greater than the quantity and value of exported milk (Table 6) (<https://www.mchamber.mk/Default.aspx?mId=19&id=29&lng=1>).

Table 6. Trade balance of milk and dairy products in North Macedonia.

Year	Export		Imports	
	Quantity in tons	Value in \$	Quantity in tons	Value in \$
2010	3.274.670	7.387.682	25.132.352	36.853.570
2011	3.727.166	9.904.373	31.472.780	52.515.519
2012	3.878.254	9.863.601	38.804.369	52.407.890
2018	5.602.300	11.402.658	36.459.259	50.326.948

The Republic of North Macedonia is an importer of milk and its products. The main importers of milk and dairy products in our country are Germany, Serbia, Slovenia, Bosnia, Greece, Bulgaria, Croatia, Montenegro, Czech Republic and France. (<https://www.mchamber.mk/Default.aspx?mId=19&id=29&lng=1>).

### **Largest milk producers/processors**

According to the latest data published by the Food and Veterinary Agency of the Republic of North Macedonia, the dairy industry in our country consists of 75 local legal entities that deal with the production, collection, and processing of milk and its products (FVA, 2021). Although it was a “Covid” year, 20 dairies managed to achieve a turnover of more than one million euros last year. The local dairy industry has survived the crisis caused by Covid-19 and has emerged stronger from the great challenge posed by the most dangerous health pandemic. Last year, 20 companies turned over more than one million euros in our market for milk and dairy products. The revenues of these enterprises amounted to 120 million euros last year. In terms of market shares, there were no major and significant changes last year, although in the first place is undeniably the largest dairy enterprise – Bitola Dairy, which has a market share of more than one-third of the total revenue from sales of the 20 largest dairy companies in the country. At the same time, it has the highest profit in absolute terms and is the only dairy enterprise with more than one million euros. The biggest player in the market last year was Trnica Dairy, which increased its sales by 98% and ended last year with a profit. The second-largest market player, Zdravje Radovo, lost the direct fight with the competitor from Kumanovo – Buçen Kozjak, which recorded a 13% increase in revenues. The subsidiary of the Croatian “Dukat”, Lactalis from Bitola, is among the four industries with the highest relative profit growth, compared to its competitors. Of the 20 companies listed in real terms, the company Deni Milk from Kocani, which, thanks to a 44% increase in revenues, has seen the greatest growth in its market share, has the smallest.

### **Dairy animals**

The world’s milk production comes almost entirely from cows, buffalo, goats, sheep, and camels. Other less common milk animals are yaks, horses, reindeer and donkeys (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>). There is considerable variation among countries and regions, as the occurrence and importance of each species vary considerably. The main factors determining the cultivation of dairy species for the dairy sector are feed, climate and water. However, socioeconomic characteristics of smallholder farmers, dietary (food) traditions, and market demand can also affect dairy cattle species. The

sustainability of farm livelihoods, communities, and ultimately water and soil resources is affected by livestock production, which is a major driver of environmental change (Pelletier and Tyedmers, 2010; Koneswaran and Nieremberg, 2008; Steinfeld et al., 2006). Globally, 81% of milk production comes from cows (81%), buffalo (15%), goats (2%), sheep (1%), and camels (0.5%). However, in developing countries, about one third of milk production comes from buffalo, goats, camels and sheep (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>). In our country, milk production is mainly based on cows, sheep and goats (State Statistical Office of the Republic of North Macedonia, News Release – Livestock Production 2020, No. 5.1.21.06, dated 29.04.2021).

Table 7. Number of livestock over the last decade.

Year	Total	Cows and heifers	Total	Sheep for breeding
2010	259.887	135.004	778.404	568.301
2011	265.299	164.537	766.631	545.214
2012	251.240	161.012	732.338	520.767
2013	238.333	154.487	731.828	530.760
2014	241.607	155.432	740.457	531.160
2015	253.442	156.699	733.510	580.840
2016	254.768	160.603	723.295	555.932
2017	255.036	153.618	724.555	565.063
2018	256.181	163.514	726.990	579.747
2019	217.790	133.740	684.558	533.393

### Production systems and milk chain

In developing countries, it is estimated that 80% to 90% of milk is produced on small farms. The production of dairy animals is relatively small, and based on low income. Smallholder dairy farming is part of a mixed farming system in which fertilizers are used to produce the first crop, dairy animals feed on grass, plant residues and cultivated fodder and supplemental feed are used only when feasible. Milk production on pastures is based on the soil and is the most important asset for existence. Milk production is usually linked to harvest, but nomadic ranchers do little or no farming and roam the countryside in search of pasture and water. Non-urban dairying is a fully market-oriented system located in or near the city limits. Peri-urban dairy producers benefit from proximity to markets, but their production is based on purchased inputs and may face difficulties with food supply and waste (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>).

Big producers do not make up a great part of national milk production. In our country, milk production is characterized by many small farms – traditional farmers (80% of the total) with a low annual milk production of 2–3 thousand liters per cow. Only 1% of farms have more than 50 cows, and these are considered as carriers of the production of high quality genetic material for the remaining farms (breeding centers) (<https://www.mchamber.mk/Default.aspx?mId=19&id=29&lng=1>). However, the importance of small dairy farms has declined precisely because of the giant corporations that control significant portions of the global dairy market and their products. The Nestlé Association, one of the major food and beverage companies globally, earned about 11 billion Swiss francs from the sale of milk in 2020. This makes milk and its products the third largest category for this multinational giant (<https://www.statista.com/topics/4649/dairy-industry/#dossierKeyfigures>).

Milk chains connect companies that distribute dairy products to the last customer, and thus, with each service, the price of the product increases. Each actor in the chain must provide the maximum added value to the product at the lowest possible cost. Creating a productive, healthy and profitable milk chain is a strong challenge in many countries, including ours. The reasons are problems in organizing a manageable milk collection and transportation system due to the small amount of milk per farm and the distance of production, seasonality, poor infrastructure, knowledge and technology deficits; lack of standard of raw milk and difficulties in setting up refrigeration facilities (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>).

### Collection and transportation

Milk is an essential food with high nutritional value that requires careful handling due to its short shelf life. It is not stable, being a perfect environment for the development of microorganisms – especially pathogenic bacteria – that cause spoilage and diseases. Processing milk makes it possible to store it and reduce the number of foodborne diseases. The shelf life of milk is affected by processes such as refrigeration (the factor most likely to affect the quality of raw milk) or fermentation. Pasteurization is a heat treatment process that can extend shelf life to several days and kill pathogenic microorganisms to the point where they do not pose a significant health risk. It can be further processed into high-quality, concentrated and easily transportable dairy products with a long shelf life, such as butter and cheese. In developing countries, small-scale producers produce most of the milk that is distributed from rural areas, and marketed in urban areas, which is challenging because of the high perishability of milk. Milk is transported by producers themselves or by milk collectors in large churning cisterns. The milk in the large containers is not adequately cooled, so the duration of transport is of

paramount importance to ensure the delivery of quality milk. On large farms, milk is usually transported in cisterns where the milk is fresh and unlikely to sour before it reaches the processing plant. Milk collection and transportation costs represent a significant portion of milk processing costs, often more than 30% (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>).

### **Institutions, associations and organizations of producers**

Food production is of strategic interest in every country. Therefore, it is important to organize food producers and represent their interests to ensure and promote food production. Dairy institutions involve dairy producer associations, regulators, service providers, market agents, nongovernmental organizations, and development partners (<https://www.mchamber.mk/Default.aspx?mId=19&id=29&lng=1>). Associations benefit producers, processors, and traders through improved revenues, market access and trading power, which results in improved production and quality of products. The dairy industry in our country lacks a variety of institutions that address the problems of developing this industry.

The milk production, collection, and processing associations have been established and operate within the Chamber of Commerce of North Macedonia. The members of associations for milk production, collection, and processing are legal entities whose primary activity is the production, collection, and distribution of milk and dairy products. The main tasks of the association are: representation and presentation of common interests before the relevant institutions and bodies, liaison with sister companies from countries in the region, review of the possibility of joint procurement of raw materials and raw materials in the production process, joint presentation at trade fairs and business forums, compliance and adaptation to EU regulations (<https://www.mchamber.mk/Default.aspx?mId=19&id=29&lng=1>).

Dairy producers can be organized into processor associations to increase their income and efficiency and to support milk collection, transportation, technology, and trading capacity. Associations are essential in enabling dairy farmers to take full advantage of milk production. The business of dairy organizations includes marketing, collection, processing, expediting access to services (e.g., finance), and providing inputs (e.g., food, loans, etc.). These organizations are critical to small-scale milk production because they provide many benefits, and improve communication and knowledge. Dairy producers' organizations have played a significant role in developing the dairy industry worldwide, but there are few such associations and organizations in our country.

### Animal health, food sources, and husbandry

Animal mortality due to diseases causes productivity losses in dairy herds worldwide, leading to significant financial losses. Mastitis and parasitical diseases reduce the system efficiency. Low milk yield or reduced milk, fertility, quality, and food processing are affected by poor animal health, which can also threaten human health (e.g., tuberculosis, brucellosis). Several factors contribute, including low knowledge of disease prevention and management, increased incidence of pathogens, and adequacy of animal health services (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>). The loss of animals can have a significant impact on the family finances of a small milk producer, who usually invests little in the health of the animals, which have numerous health problems. Dairy animals adapted to local environmental and climatic conditions can significantly reduce animal welfare and health problems and are resistant to endemic parasites and diseases. One of the most important factors limiting milk production is poor digestibility and nutrition of animals, mainly due to plant residues and poor pasture quality with few vitamins, minerals and nitrogen (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>).

Moving livestock to pasture is a common practice in all developing countries. The use of supplementary feeds (foods rich in energy and protein) is essential for dairy animals because milk production is a process that consumes much energy and availability is low. Small-scale milk producers usually cannot feed. Small-scale milk producers depend mainly on seasonal quantity and quality of natural forage due to the high cost of conventional supplemental feeds such as minerals, oilseeds and grain-based concentrates. Through selective breeding and reproductive control, dairy producers can improve productivity and income from milk production (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>). Breeding success is influenced by factors such as genetic characteristics and suitability, production system and environment, socioeconomic conditions of producers, and feed. Reproductive performance (conception rates and birth intervals) can be improved by using appropriate genotypes, valuable traditional knowledge of breeds and their management for breeding practices, and also by using knowledge of genetics and breeding from scientific institutions. Even if these are not formalized or written down, they have breeding objectives and strategies. In developing countries, large-scale dairies routinely use artificial insemination by producing breeding males or by having small-scale farmers or individual producers use artificial insemination, paying increasing attention to the breeding process. Kumar and Meena (2021) reported that the socioeconomic status of farmers is mainly responsible for their decision regarding animal health services.

### **Financial support for the dairy industry**

The Ministry of Agriculture, the Agency for Financial Support to Agriculture and Rural Development and the Government of the Republic of North Macedonia, through their policies, continue to create favorable measures and policies that will support the growth and development of the agricultural sector in the country, including the production of milk and its products. Regarding financial support to the dairy industry, the latest data show that the Agency for Financial Support to Agriculture and Rural Development transferred 255.9 million denars to the accounts of 7,347 entities ([http://www.ipardpa.gov.mk/Root/mak/default\\_mak.asp](http://www.ipardpa.gov.mk/Root/mak/default_mak.asp)). It refers to the payment to farmers who produced and supplied sheep's, goat's and cow's milk. Farmers who produced and delivered cow's milk received a support of 3.5 denars per liter, while all those who produced goat and sheep milk received a financial support of 4.5 denars per liter of delivered milk. According to the Regulation on Direct Payments for 2021, the criteria for direct charges related to livestock production include: consumers, milk producers and deliverers of cows, sheep and goats. It establishes the requirements for direct payments related to livestock production (Regulation 2021, Official Gazette of RNM No. 12/2021).

The beneficiaries of this measure are agricultural cooperatives – producers of cow's, sheep's and goat's milk registered as breeders of cattle, sheep and goats in the Register of breeders of certain animal species (cattle, sheep and goats). The investments and financial support are of particular interest in shaping the policies of the Government and the Ministry of Agriculture, Forestry and Water Economy as the milk and dairy industry is one of the most critical segments in the agro-industrial complex. Financial support for the livestock supply measure was significantly increased. Furthermore, nearly 1.5 million euros were allocated for this purpose (<http://www.mzsv.gov.mk/Events.aspx?IdRoot=50&IdLanguage=3&News=504>). This measure is essential to improve the genetic potential, which will ultimately affect the quality of the final product, while other measures are foreseen to support this sector. However, the measures are also available from the IPARD2 program, which provides an opportunity for investments in the construction or reconstruction of dairies, farms and the supply of equipment (<http://www.mzsv.gov.mk/Events.aspx?IdRoot=50&IdLanguage=3&News=504>).

Numerous mechanisms are used within the EU to preserve the dairy industry in times of widespread trade turmoil. These intervention measures aim to address the possible market imbalance by protecting consumers, producers, and processors within the dairy industry. The European Union is estimated to be the largest exporter of milk and dairy products globally. However, there are numerous policies within the Union, to promote research, innovation, and improve dairy product

standards. Past and current trends at the European and global levels in the dairy industry are analyzed by European authorities on issues such as production costs and market prospects for dairy products ([https://ec.europa.eu/info/food-farming-fisheries/animals-and-animal-products/animal-products/milk-and-dairy-products\\_en](https://ec.europa.eu/info/food-farming-fisheries/animals-and-animal-products/animal-products/milk-and-dairy-products_en)).

### Economics of the dairy industry

Dairy milk production provides a systematic financial income and is based on the quality of milk composition, hygienic standards and seasonality. Additionally, income includes the sale of slaughtered animals and other income from dairy farming, such as the sale of manure. The dairy industry provides numerous financial gains that are not traded, including manure for farm use as organic manure (in some agricultural systems, manure is the only source of nutrients for plant productivity). Dairy animals are viewed as a form of capital investment or a means of savings to sell in times of need, despite the risk of theft or death. Since small-scale dairy producers generally use domestic labor, small-scale milk production costs (per unit of milk produced) are often similar to those of large-scale dairies. Retail milk production depends little on family labor, and labor is occasionally hired. Small-scale milk production provides business opportunities through the dairy industry, including small-scale processing plants and other direct and indirect services, with particular emphasis on feed having a significant impact on the dairy economy. Between these strategies and solutions, sustainable intensification and agroecology in ruminant farming systems have been proposed as environmentally safe, economically feasible, and socially just (Dumont et al., 2018).

### Social and gender issues and the dairy industry

In developing countries, more than 80% of milk is produced on small farms that provide employment to more than 750 million people worldwide (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>). Less labor-intensive production systems and a more technology-intensive dairy sector provide less employment per milk production unit in developed countries, while farm income increases in developing countries due to market-oriented opportunities. In addition, the dairy sector provides more employment opportunities than other food chains (<https://www.fao.org/dairy-production-products/socio-economics/dairy-development/en/>).

In many rural communities, as in our society, women traditionally play an important role in the production of milk – feeding and milking – processing and marketing dairy products. While they usually do not earn money from their work,

the milk they sell allows them to buy food, clothing and other necessities and to pay for their children's education and basic needs. Nevertheless, women's participation in milk production is generally decreased because milk production plays an important role in providing family income. Basically, strategies for sustainable farming practices have been proposed as an innovative process to increase animal welfare and social acceptance of the dairy sector (Padel et al., 2015).

### **Challenges of the dairy industry**

Today, the dairy industry faces various interrelated challenges in the three main pillars of sustainability: economic, social and environmental. The dairy sector within the EU faces various socioeconomic challenges, including market price fluctuations, high labor costs, and demographic characteristics of the population in terms of age. All farm systems are directly related to the climate and the function of a healthy natural environment. The European dairy industry is both a victim and a cause of climate change (Alvez et al., 2013). Farming within the EU is responsible for about 10% of greenhouse gas (GHG) emissions coming from the economy (Bas-Defossez et al., 2019). Social understanding and recognition of environmental impacts, animal welfare and health issues are constantly increasing, resulting in growing consumer demand for standards of healthy, sustainable products and high levels of animal welfare.

The EU assumes that by 2050 the EU dairy industry will have found the balance between the three pillars of sustainability: environmental, economic and social (Bas-Defossez et al., 2019). This will enable the industry to operate safely and meet consumer needs and demand for healthy and sustainable products supported by high standards of animal welfare. The dairy sector will be profitable, with capital distributed across supply and value chains, and a large attractive employer. In many developing countries, dairy progress is limited by livestock issues, transportation, processing, refrigeration, and marketing. In addition, small-scale dairy farmers cannot manage their farms as businesses. They have little access to services such as training, education, health, and lending, and have little or no investment capital due to the small size of their herds. This is the case with small-scale dairy producers in our country. As the modern dairy sector in Europe occupies one third of the agricultural land in the EU and increases its dependence on concentrated feed, the adoption of new technologies in nutrition, genetics, and herd management is accelerating, and cows are mostly located in modern facilities with limited or no access to pasture (EC, 2021; Zorn and Zimmert, 2022; Bórawska et al., 2020; Barkema et al., 2015).

## Conclusion

Depending on cultural and social circumstances, market demand, available milk processing technologies and dietary habits, dairy products vary considerably from region to region in the same area. Factors driving the development of the dairy industry include changes in production, demand, communication and transportation technology, improved on-farm productivity, and a more effective dairy production chain. It is essential to add merit to every activity in the dairy production chain. Establishing active producer associations and credible milk chains is vital for sustainable development. In the Republic of North Macedonia, milk production is characterized by many traditional farmers with small farms (75% of the total) with low productivity (1–3 cows) and low milk production annually (2–3000 liters per cow). Insignificant is the number of farms with annual milk production of about 5 thousand liters per cow or more than 20 cows (about 3% of the total) and that of farms with more than 50 animals is around 1%. Therefore, we can conclude that milk production in our country has an overall downward trend. Dairy production in our country is characterized by low productivity and an incomplete identification system that makes it challenging to monitor and control diseases in domestic animals, which significantly affects the quality and safety of milk.

Primary production is still a fundamental problem that concerns the provision of cheap food, more modern breeding conditions, improving the breed composition of livestock, improving the microbiological quality of milk and others. The specific way of milk production, distribution and transportation makes it difficult to implement the concept of milk control, and the method of control itself is quite expensive. Many dairies do not meet basic hygienic and sanitary requirements, and it is not uncommon to encounter unregistered processing plants. There is a violation of existing standards and procedures for proper technological methods, inadequate control of incoming material and performance in its factory in terms of product quality and safety. Most milk is traded through informal channels without regulatory licensing. In our country, the situation is similar, especially in rural areas. The dairy trade improves revenues, creates jobs in processing, marketing and distribution, and contributes to food security.

To achieve poverty alleviation, food security and economic growth, the success of dairy farming due to milk production is a powerful tool. It contributes with a systematic source of food with nutritional value, increases resource utilization, creates jobs inside and outside the farm, opens opportunities for women and the most affected social groups, and provides financial stability and social status. The Republic of North Macedonia is an absolute importer of milk and its products. The quantity and monetary value of imported milk and its products are many times higher than the quantity and value of exported milk. Today, the global

dairy industry faces various interrelated challenges in the three main pillars of sustainability: economic, social and environmental. The dairy sector faces multiple socioeconomic challenges, including fluctuating market prices, high labor costs, and demographic characteristics of the population in terms of age. All farm systems are directly related to the climate and the functioning of a healthy natural environment. Social understanding and awareness of environmental impacts, animal welfare and health issues are constantly increasing, resulting in growing consumer demand for standards of healthy, sustainable products and high levels of animal welfare. In conclusion, for the dairy industry to be sustainable, all activities should be carried out in partnership with the industry (along the value chain), policymakers and a wide range of stakeholders, including consumers themselves.

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## ODRŽIVOST I PERSPEKTIVE INDUSTRIJE MLEKA U SEVERNOJ MAKEDONIJI

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### R e z i m e

Predmet ovog pregleda je industrija mleka u Republici Severnoj Makedoniji. Ova industrija može doneti mnoge ekonomske i socijalne koristi društvu i privredi. U radu je dat opis i analiza postojećeg stanja u industriji mleka u našoj zemlji. Analiziraju se stope proizvodnje mleka u našoj zemlji, uz uporedni pristup stopama proizvodnje na svetskom i evropskom nivou. U Republici Severnoj Makedoniji proizvodnju mleka karakteriše veliki broj tradicionalnih malih poljoprivrednika (80% od ukupnog broja) sa 1–3 krave i niskom godišnjom produktivnošću mleka (2–3 hiljade litara po kravi). Broj velikih specijalizovanih gazdinstava sa više od 15 krava (oko 3% od ukupnog broja) sa oko 5 hiljada litara godišnje proizvodnje mleka po kravi je minimalan. Samo 1% svih gazdinstava ima više od 50 krava. Republika Severna Makedonija je apsolutni uvoznik mleka i mlečnih proizvoda. Količina i novčana vrednost uvezenog mleka i mlečnih proizvoda višestruko su veće od količine i vrednosti izvezenog mleka. Danas se industrija mleka suočava sa različitim izazovima u okviru tri glavna stuba održivosti: ekonomskom, socijalnom i ekološkom, uključujući fluktuirajuće tržišne cene, visoke troškove rada i demografske karakteristike stanovništva u pogledu starosti. Da bi mlečna industrija bila održiva, sve aktivnosti treba da se sprovode u partnerstvu sa industrijom (duž lanca vrednosti), kreatorima politike i širokim spektrom zainteresovanih strana, uključujući potrošače.

**Ključne reči:** industrija mleka, finansijska podrška, kvalitet mleka, standardi za mlečne proizvode.

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## BENEFICIAL EFFECTS OF SILICON FERTILIZERS ON INDICATORS OF SEED GERMINATION, GRAIN YIELD OF BARLEY AND SOYBEAN AND SILAGE CORN BIOMASS

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**Abstract:** The aim of the study was to determine the optimal doses and methods of the application of silicon fertilizer in barley, silage corn, and soybean under the climatic conditions of Ukraine. A series of laboratory and field experiments were carried out, as along with statistical and analytic data processing. The small-plot field experiment was conducted on chernozem podzolic soil at the NSC “Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky” (Kharkiv region, Forest-Steppe of Ukraine). The laboratory experiments were set up in Petri dishes using a preparative form of different concentrations (0%, 0.5%, 0.75% and 1.0%) of potassium silicate for their assessment of the germination indicators of barley and corn seeds. The significant stimulating effect of potassium silicate on the germination capacity and germination energy of barley and corn seeds was shown, and the optimal concentration of the solution for pre-sowing seed treatment was established. A high positive correlation was determined between the amount of  $\text{SiO}_2$  application and the yield of the studied crops ( $P < 0.01$ ;  $R = 0,7479-0,8682$ ). The optimal levels of the pre-sowing application of  $\text{SiO}_2$  into the soil were established to obtain maximum crop yields on chernozem podzolic soil (105 kg  $\text{SiO}_2 \text{ha}^{-1}$  for barley, 92 kg  $\text{SiO}_2 \text{ha}^{-1}$  for silage corn, and 76 kg  $\text{SiO}_2 \text{ha}^{-1}$  for soybean). Also, for each of the studied crops, the most optimal methods of the application of silicon fertilizers were determined, which will improve their productivity on chernozem soils.

**Key words:** silicon, plant nutrition, crop productivity, mineral fertilizer.

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## Introduction

Degradation of soil cover, environmental pollution, decline in the quality of agricultural products, increased frequency of extreme weather conditions, and deficiency of energy resources require the widespread introduction of new, environmentally adaptable, and at the same time, highly efficient methods of farming. Much of the degradation of agricultural land and the decline in the quality of agricultural products is associated with unbalanced fertilization and plant nutrition (Aristarkhov and Mineev, 2000). However, in comparison with the deep theoretical substantiation of the optimization of nitrogen-phosphorus-potassium nutrition in the soil-plant continuum, the issues of mesoelements (S, Si, Mg) have received much less attention in agrochemical studies. Nevertheless, it is well known that all elements of mineral nutrition are closely related to each other through participation in common processes, but the role of each of them is strictly specific.

The biological value of nutrients such as silicon (Si) has been known for over 150 years, but its role in plant nutrition, especially under stress conditions, has only been studied carefully in recent decades (Liang et al., 2015). Less interest among researchers could be explained by the fact that silicon does not belong to strictly essential elements for plants, since they can perform their life cycles without it (Epstein, 1999; Liang et al., 2015). In addition, Si is the second most abundant element in the Earth's crust and accounts for 27.7% of the total soil mass. Its total content in clay soils ranges from 200 to 300 g per kg, in sandy soils – 450–480 g per kg (Haynes, 2014; Liang et al., 2015; Meharg and Meharg, 2015). However, plants absorb only soluble forms of Si – the monomer of silicic acid, and its anions (Ma et al., 2001). Silicon is present in plants in amounts equivalent to those macronutrient elements such as calcium, magnesium, and phosphorus (Epstein, 1999), and in grasses often at higher levels than any other inorganic constituent, and the removal of this element by the crop yield ranges from 20 to 700 kg per ha, which is close to removing nitrogen, phosphorus, and potassium (Bocharkova and Matichenkov, 2012). Silicon is a structure-forming element in soil; therefore, its constant removal leads to a deficit that reduces the natural protective properties of agricultural plants (Bocharkova and Matichenkov, 2012).

Numerous studies show that Si improves the growth, yields and quality of crops as well as photosynthesis, nitrogen and phosphorus utilization in soil (Liang et al., 2007, 2015; Cooke and Leishman, 2011; Guntzer et al., 2012; Van Bockhaven et al., 2013; Cuong et al., 2017; Jiang-xue Long et al., 2018). The role of silicon is especially important in increasing plant resistance to abiotic and biotic stresses; therefore, Fauteux et al. (2006), Chain et al. (2009), Nwugo and Huerta (2011), Epstein (2009), Van Bockhaven et al. (2013) believe that its effectiveness is limited exclusively by stressful conditions. The optimization of silicon nutrition

is considered to help plants adapt to drought, high temperatures, frost, radiation, ultraviolet radiation, and chemical stresses caused by salinity, metal toxicity, nutrient imbalances, etc. (Epstein, 1994; Hattori et al., 2005; Ma, 2004). However, long-term studies have also proved the effectiveness of silicon fertilization under non-stressful conditions (Tama and Ma, 2008; Detmann et al., 2012, 2013). The use of silicon as a fertilizer helps to improve the yield components of wheat, rice, soybean, corn, rapeseed, and sugar beet by improving photosynthetic activity, increasing the efficiency of using macronutrients and soil moisture (Maghsoudi et al., 2015; Amin et al., 2016; Artyszak et al., 2016; Artyszak and Kuci'nska, 2016; White et al., 2017; Walsh et al., 2018). It is also important that the use of silicon is safe for the natural environment, and that it can be used in organic farming, which has become highly important.

So, we can assume that silicon has the characteristics of an essential nutrient for plant growth and, according to Ma et al. (2001), it can be included in the list of the main elements for increasing the productivity of grain crops. In 1999, the first scientific conference on "Silicon and Agriculture" was held in the United States and this gave an impetus for the global fertilizer market and agricultural production in general to pay attention to silicon-containing fertilizers (Jiang-xue Long et al., 2018).

Since the use of silicon fertilizers significantly improves the growth and development of agricultural crops, increases the level of their productivity, and improves the quality of the products obtained under stressful conditions, it is relevant to determine the optimal doses and methods of applying silicon fertilizers in the soil and climatic conditions of Ukraine. It is also important to clarify the possibility of their combination with mineral fertilizers for crops that occupy an important place in the structure of sown areas in Ukraine. The aim of this study was to determine the optimal doses and methods of applying silicon fertilizers under barley, corn and soybean in the soil and climatic conditions of Ukraine.

## Material and Methods

The studies were carried out with a preparative form of potassium silicate with a mass fraction of silicon dioxide – 21.3% and potassium oxide – 8.3% under the conditions of laboratory experiments and a small-plot field experiment.

The aim of the laboratory experiments was to determine the optimal concentration of the working solution of potassium silicate for pre-sowing seed treatment. Laboratory studies assessed the effect of potassium silicate on the germination of barley and corn seeds according to the following indicators: germination capacity, germination energy, and germination synchronization index. The course of this experiment included: 100 seeds of barley and corn, previously kept for 8 hours in solutions of potassium silicate at four concentrations (0%, 0.5%,

0.75%, and 1.0%) with four replicates laid out on the filter paper moistened with distilled water in the Petri dishes. In the control variant, seeds were soaked in distilled water. The dishes were kept in a lighted laboratory cabinet at a constant air temperature of 25°C for 7 days and controlled by the humidity of the filter paper. An assessment of the germination energy (GE) was carried out on day 4, and of the germination capacity (GC) on day 7 following the placement of seeds on the filter paper in the Petri dishes. The germination synchronization index (GSI) was determined according to a modified methodological approach by Primack (1980) – and calculated using the formula:

$$GSI = \frac{GC}{D} \quad (1)$$

GSI – germination synchronization index, %;

GC – germination capacity, %;

D – number of days of germination.

Additionally, the primary root length was measured in 10 normal seedlings randomly obtained after the germination capacity calculation (on the 7<sup>th</sup> day).

The field experiment was conducted in 2020 at the experimental site of the NSC “Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky” (State Enterprise “Experimental Farm Grakivske” Kharkiv district of Kharkiv region, Ukraine, geographical coordinates: 49°46'N lat., 39°40' E long). The experiments were established on chernozem podzolic heavy loamic soil (Luvic Chernozem according to WRB) with the following characteristics: pH<sub>KCl</sub> – 6.0; organic carbon content determined by the Tyurin method – 2.8%; the content of mobile phosphorus and potassium determined in acetic acid extract – 138 and 90 mg kg<sup>-1</sup>, respectively. The sum of precipitation during the research period was properly distributed, and it amounted to 417 mm from April to September (Table 1).

Table 1. Weather conditions during the growing season of 2020.

Months	Air temperature, °C		Precipitation, mm	
	study period	average annual data	study period	average annual data
April	12.1	11.0	46.0	34.5
May	15.4	17.9	148.0	38.9
June	20.1	21.9	54.0	42.2
July	22.6	21.9	92.0	50.2
August	22.3	20.3	63.0	42.0
September	14.0	16.0	14.0	19.0
April–September	17.75	18.2	417.0	226.8

In the field experiment, three agricultural crops were grown according to the technology traditional for the forest-steppe zone of Ukraine. Spring barley variety Helios was sown on April 5, 2020, and the crop was harvested on July 3, 2020. The seed rate was 450 seeds/m<sup>2</sup>, and the row spacing was 15 cm. Silage corn hybrid DN Anshlah (FAO 420) was sown on April 27, 2020, and harvested on August 20, 2020. The seed rate was 80 thousand seeds/ha, and the row spacing was 70 cm. Soybean variety Baika was sown on May 7, 2020, and harvested on September 18, 2020. The seed rate was 750 thousand seeds/ha, and the row spacing was 15 cm.

The scheme of field experiments for each crop included 5 treatments:

- 1) Basal fertilization (control) (different for each crop);
- 2) Basal fertilization + potassium silicate (banding of 60 kg SiO<sub>2</sub> ha<sup>-1</sup>);
- 3) Basal fertilization + potassium silicate (banding 120 kg SiO<sub>2</sub> ha<sup>-1</sup>);
- 4) Basal fertilization + potassium silicate (banding 60 kg SiO<sub>2</sub> ha<sup>-1</sup>) + foliar application (30 kg SiO<sub>2</sub> ha<sup>-1</sup>);
- 5) Basal fertilization + foliar application of 30 kg SiO<sub>2</sub> ha<sup>-1</sup>.

The experiments were conducted as small plot research trials with six replications in a completely randomized design. The rate of the application of the basal fertilization for barley and corn was N<sub>40</sub>P<sub>40</sub>K<sub>40</sub>, and for soybean – N<sub>90</sub>P<sub>60</sub>K<sub>60</sub>. The basal fertilization was applied in the form of nitroammophoska (16:16:16) and ammonium nitrate broadcast under pre-sowing cultivation, potassium silicate – during sowing of agricultural crops into the soil locally in the band at a distance of 3–4 cm to the side and 10–12 cm below the sowing row. The foliar applications of potassium silicate were made to spring barley in the tillering phase, to corn – in the phase of 6–8 leaves, to soybean – in the phase of the third to fifth nodes (V3 to V5).

Soybean grain yield was calculated at 13–14% moisture content and barley grain yield at 14–17% moisture content. Silage corn was harvested at milk-wax maturity (biomass weight was measured).

All the data were analyzed using STATISTICA 13.5.0.17. The least significant difference (LSD) test for treatment parameters was done after performing ANOVA, at the P<0.05 significance level. Quadratic regression equations were also developed to describe the performance of silicon fertilizers for each crop.

## Results and Discussion

Seed treatment with a silicate solution provides plants with additional silicon at the seed germination phase. Kozlov et al. (2015) found that the change in the quality indicators of grain crops is significantly affected by the dose of silicon, in particular, the concentration of the working solution in seed treatment.

The results of our study showed that the indicators of germination capacity and germination energy of barley and corn seeds were higher in all seed treatments with potassium silicate solution than in the control, except for germination energy in barley (Table 2). However, no significance was observed in 1.0% working solution treatment compared to the control. Increasing the concentration of potassium silicate solution above 0.75% was not effective for the pre-sowing seed treatment. The treatment with a 0.5% potassium silicate solution also did not differ significantly from the control, which indicates an insufficient stimulating effect of this concentration.

Table 2. Parameters of germination of seeds treated with a potassium silicate solution of various concentrations (laboratory experiment).

Experiment options (concentration of potassium silicate solution)	Germination capacity (%)	Germination energy (%)	Germination synchronizatio n index (%)	Root length (cm)
<i>Barley</i>				
Control (without treatment)	85.0	85.0	12.1	8.4
0.5%	90.0	85.0	12.9	8.8
0.75%	100.0	95.0	14.3	9.4
1.0%	87.5	85.0	12.5	8.7
SE	4.9	3.1	0.7	0.1
<i>LSD</i> ( <i>p</i> ≤ 0.05)	15.6	10.1	2.2	0.3
<i>Corn</i>				
Control (without treatment)	87.5	87.5	12.5	8.8
0.5%	91.6	91.6	13.1	9.2
0.75%	100.0	100.0	14.2	10.5
1.0%	95.8	87.5	13.7	9.7
SE	3.4	3.4	0.5	0.2
<i>LSD</i> ( <i>p</i> ≤ 0.05)	10.8	10.8	1.6	0.6

The highest values of germination capacity and germination energy, which were 10–15% higher for barley seeds and 12.5% higher for corn seeds compared to the control, were determined when seeds were treated with a 0.75% potassium silicate solution. The changes in the germination synchronization index of barley and corn seeds, depending on their treatment with a potassium silicate solution at various concentrations, had the same character as for the indicators of germination and germination energy. Therefore, increasing the concentration of the working solution to 1.0% of potassium silicate did not improve all indicators of seed germination.

Germination and germination energy are the main indicators of seed quality that directly affect the yield of agricultural crops and are especially important under

conditions of the increasing frequency of droughts and other abiotic stresses associated with climate change (Pati et al., 2016).

Another indicator of the effect of potassium silicate on seed germination, which was determined in a laboratory experiment, is root length. The optimization of Si nutrition increases root mass and volume and increases total and adsorbing surfaces (Matichenkov, 1996). Since the root system is responsible for the absorption and transport of nutrients and water to plants, the normal growth and development of the underground part of the plant are extremely important for the further formation of a crop. The length of corn roots on the 7<sup>th</sup> day of seed germination under all treatments ranged from 8.8 cm to 10.5 cm, and for barley this indicator was 8.4–9.4 cm. Root length of corn was increased by 2.6% and in barley by 9.4% compared to the control. The most pronounced beneficial effect was obtained when seeds were treated with a potassium silicate solution at a concentration of 0.75%.

The positive effect of treating corn and barley seeds with potassium silicate solutions on their germination indicators, confirmed by the data in Table 2, would contribute to the formation of a developed root system of the plants and to an increase in the water content in the tissues in case of soil water deficit in spring. Numerous studies show that monosilicic acids increase the germination of cereal seeds, accelerate the formation of rice, and promote the ripening of citrus fruits (Savant et al., 1997; Matichenkov et al., 1999). The stimulation of the growth of primary roots of crops discovered correlates with information on the optimization of silicon nutrition of plants, which leads to an increase in the total mass of roots (Matichenkov, 1996), their branching (Matichenkov et al., 1999), the total and working adsorbing surface (Kudinova, 1975), and increased root respiration (Yamaguchi et al., 1995).

In the field experiment, in order to determine the effect of silicon fertilizer on the productivity of barley, soybean and silage corn, potassium silicate was applied locally into the soil and foliarly. The use of silicon fertilizer significantly influenced the productivity of spring barley (Table 3). In all studied treatments, a significant increase in the grain yield and total aboveground biomass relative to control was obtained.

The increase in total barley biomass ranged from 20% to 53% while the percentage of grain in total biomass was 48.2–51.7. The highest increase in both grain yield and aboveground biomass was obtained under treatment with 120 kg SiO<sub>2</sub>/ha. Surprisingly, under treatment with combined silicon soil and foliar application, both grain yield and aboveground biomass were significantly lower in comparison to the treatment with corresponding soil fertilization (Table 3).

In our opinion, an increase in the total biomass of barley plants, first of all, may be due to an increase in the growth of the root system at the initial stages of plant ontogenesis. In particular, the use of silicon-containing fertilizers for barley

in the experiments of Balakhnina et al. (2012) and Włodarczyk et al. (2019) stimulated the growth of shoots and roots of barley, leading to a significant increase in biomass under optimal growing conditions. The application of readily soluble silicon increases the availability of Si in the soil and also stimulates the absorption of more Si from the soil solution (Pati et al., 2016). As noted by Ahmad et al. (2016), the addition of potassium metasilicate ( $K_2SiO_3$ ) to the soil promotes the concentration of  $K^+$  ions in plant shoots, which helps to maintain water potential under drought conditions.

Table 3. The effect of potassium silicate soil and foliar application on the aboveground biomass and grain yield of spring barley.

Field experiment treatments	Aboveground biomass, (t $ha^{-1}$ )	Grain yield, (t $ha^{-1}$ )	Percentage of grain from biomass yield (%)
Basal fertilization (control)	4.50	2.52	56.0
Basal fertilization + potassium silicate (banding 60 kg $SiO_2 ha^{-1}$ )	6.60	3.18	48.2
Basal fertilization + potassium silicate (banding 120 kg $SiO_2 ha^{-1}$ )	6.90	3.40	50.7
Basal fertilization + potassium silicate banding (60 kg $SiO_2 ha^{-1}$ ) + foliar application (30 kg $SiO_2 ha^{-1}$ )	5.69	2.94	51.7
Basal fertilization + foliar application (30 kg $SiO_2 ha^{-1}$ )	5.40	2.68	49.6
SE	0.72	0.31	-
LSD( $p \leq 0.05$ )	0.19	0.10	-

Basal fertilization (40 kg  $N ha^{-1}$  + 40 kg  $P_2O_5 ha^{-1}$  + 40 kg  $K_2O ha^{-1}$ ); SE, standard error.

Based on the experimental data obtained, a quadratic regression equation was developed ( $y = 2.343 + 0.021*x - 0.0001*x^2$ ,  $P < 0.01$ ), where the top of the curve with an abscissa value of 105 kg  $ha^{-1}$  was the highest point on the quadratic function graph (Figure 1). This value can be considered as the optimal level of the potassium silicate application rate, which provided the highest grain yield of 3.44 t  $ha^{-1}$ .

It should be noted that, in addition to the direct effect of silicon on the plant organism, an increase in the yield of agricultural crops may be due to its indirect effect on the improvement of phosphorus nutrition of plants. A solution of silicic acid can displace phosphorus from sparingly soluble phosphates that are not available to plants, and this leads to an increase in the concentration of phosphate ions in the soil solution. In addition, the silica hydrogel can adsorb phosphorus ions contained in the soil solution, thereby preventing the chemical binding of phosphate ions by the soil (Cuong et al., 2017).

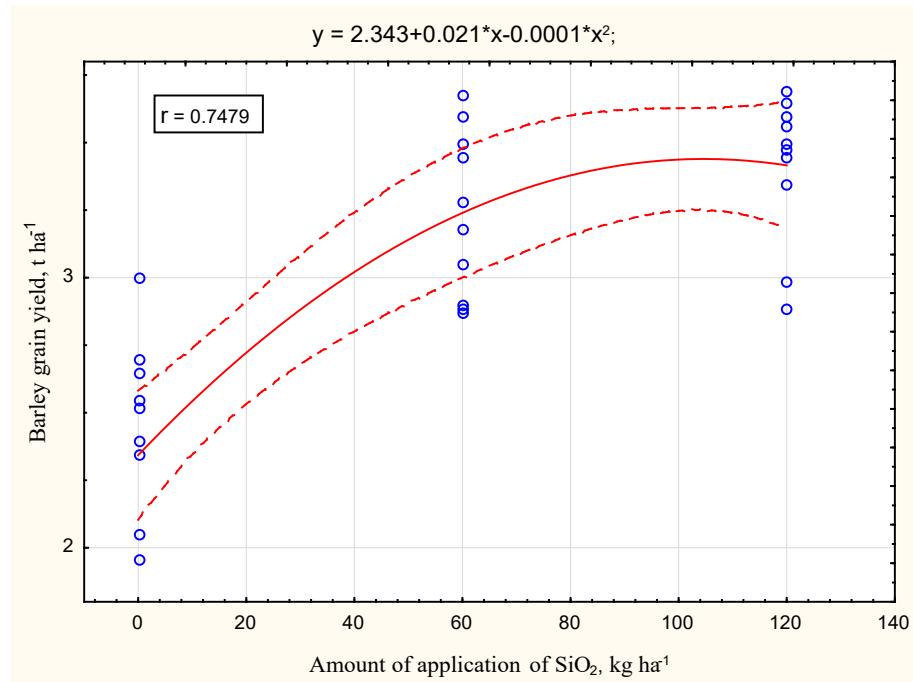


Figure 1. The effectiveness of silicon fertilizers for spring barley.

Field studies have also proven the beneficial effects of potassium silicate on silage corn and soybean yields. In all treatments with silicon fertilizer, significant increases in yield were obtained compared to the control: for silage corn – from 16% to 57.3%, for soybean – from 54% to 138% (Table 4). Treatments with 120 kg SiO<sub>2</sub> ha<sup>-1</sup> and combined potassium silicate soil and foliar application gave the highest increase in the biomass of corn and barley grain yield, respectively.

Quadratic regression models can be used to determine the optimal levels of silicon fertilization. The maximum of the quadratic function of the dependence of the silage corn biomass on the SiO<sub>2</sub> dose was 92 kg SiO<sub>2</sub>ha<sup>-1</sup> (Figure 2A), while the maximum of the quadratic function for soybean was 76 kg SiO<sub>2</sub>ha<sup>-1</sup> (Figure 2B). At these levels of potassium silicate application, the silage corn biomass was 36.3 t ha<sup>-1</sup>, and gain yield of soybean – 2.4 t ha<sup>-1</sup>. A further increase in the SiO<sub>2</sub> dose is not justified.

At the same time, the combined soil and foliar application of silicon fertilizers in soybean proved to be more effective than soil application only, which made it possible to obtain the maximum payback of 1 kg SiO<sub>2</sub> by grain yield (Table 4).

Table 4. The effect of potassium silicate soil and foliar application on the biomass of silage corn and the grain yield of soybean.

Field experiment treatments	Silage corn		Soybean	
	Yield of silage biomass, t ha <sup>-1</sup>	Payback of 1 kg of active substance potassium silicate by 1 centner of yield	Grain yield, t ha <sup>-1</sup>	Payback of 1 kg of active substance potassium silicate by 1 centner of yield
Basal fertilization (control)	22.53	-	1.33	-
Basal fertilization + potassium silicate (banding 60 kg SiO <sub>2</sub> ha <sup>-1</sup> )	33.24	1.78	2.17	0.13
Basal fertilization + potassium silicate (banding 120 kg SiO <sub>2</sub> ha <sup>-1</sup> )	35.40	1.08	2.43	0.13
Basal fertilization + potassium silicate banding (60 kg SiO <sub>2</sub> ha <sup>-1</sup> ) + foliar application (30 kg SiO <sub>2</sub> ha <sup>-1</sup> )	30.71	0.91	3.10	0.20
Basal fertilization + foliar application (30 kg SiO <sub>2</sub> ha <sup>-1</sup> )	26.12	1.2	2.01	0.23
<i>LSD(p ≤ 0.05)</i>	5.87	-	0.64	-

Basal fertilization: for corn – 40 kg N ha<sup>-1</sup> + 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> + 40 kg K<sub>2</sub>O ha<sup>-1</sup>; for soybean – 90 kg N ha<sup>-1</sup> + 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> + 60 kg K<sub>2</sub>O ha<sup>-1</sup>; SE, standard error.

A little-studied aspect of the effect of silicon fertilizers on plant growth and development is the effective combination with optimal doses of NPK. According to the results of Jawahar et al. (2017), when growing corn, it was better to combine the application of monosilicic acid into the soil with optimal doses of NPK. Our results are consistent with these studies in that the amount of basal fertilizers applied for each crop was sufficient to obtain a positive crop response from the combined application of silicon fertilizers and NPK.

A similar close correlation between the amount of SiO<sub>2</sub> and the yield of soybean ( $P < 0.01$ ,  $R = 0.81$ ) in our field experiment indicates that the mechanism of the positive action of silicon may be similar. Other researchers also point to the agronomic efficiency of using silicon fertilizers for growing soybean under various soil and climatic conditions. For instance, an increase in soybean yield by 7.5–13.6% compared to control under silicon fertilizer was determined in China (Liang et al., 2015) and by 21% in Poland (Ciecielski, 2016). Consequently, the inclusion of silicon in the system of fertilization of agricultural crops in Ukraine can be considered a fairly reasonable step, although this does not exclude the need for a deeper study of the mechanisms of action of Si in various forms of silicon fertilizers and methods of their application.

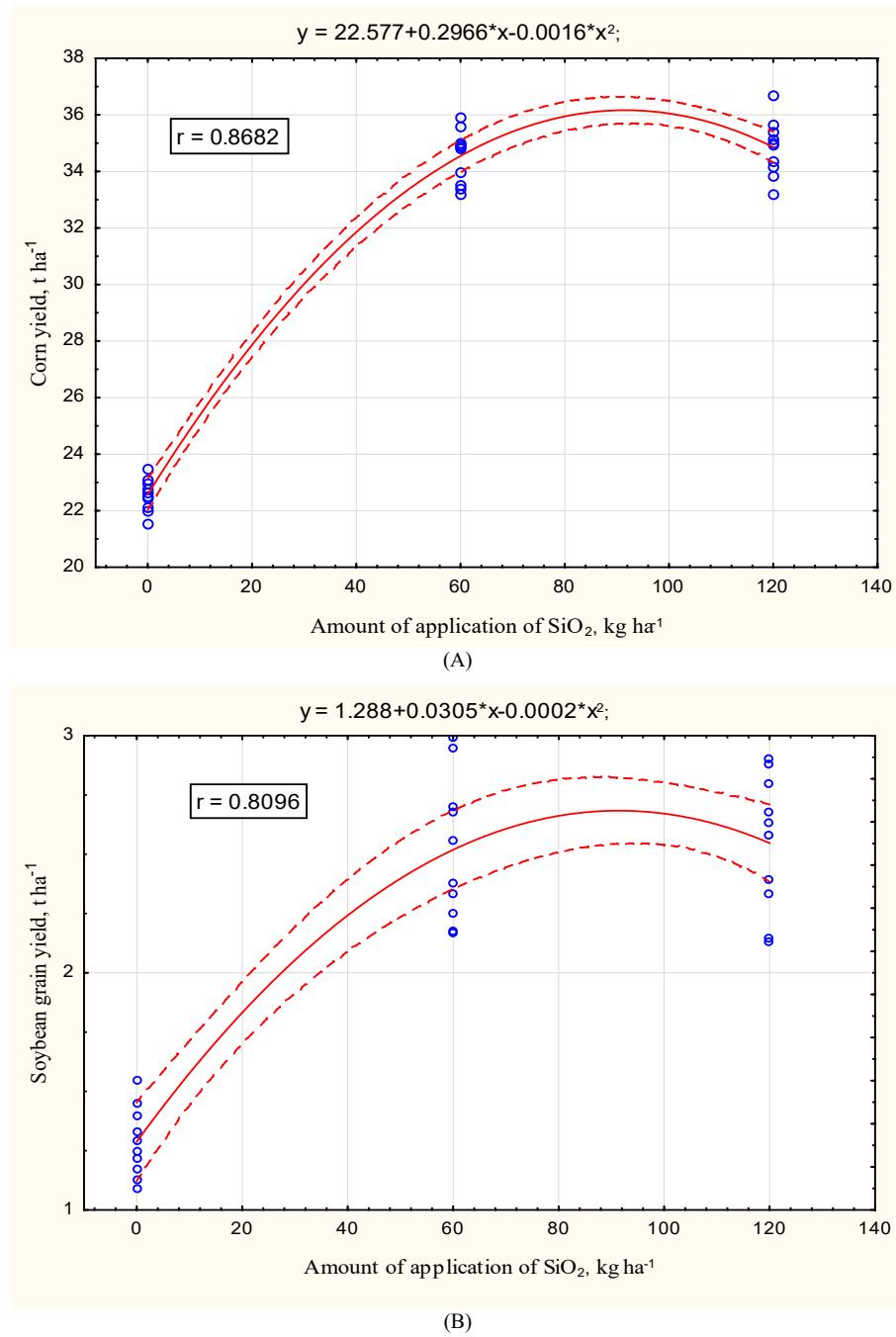


Figure 2. The efficiency of the application of silicon fertilizers for corn (A) and soybean (B).

## Conclusion

The growth-stimulating effect of potassium silicate on the indicators (germination capacity, germination energy, and germination synchronization index) of barley and corn seeds was found; the optimal concentration of the  $\text{SiO}_2$  solution for pre-sowing seed treatment was 0.75%. The application of potassium silicate during sowing into the soil contributed to an increase in the aboveground mass and grain yield of spring barley, biomass yield of silage corn and soybean grain. The highest effect of 1 kg  $\text{SiO}_2$  for these crops was determined at application rates of 105 kg  $\text{SiO}_2 \text{ ha}^{-1}$ , 92 kg  $\text{SiO}_2 \text{ ha}^{-1}$  and 76 kg  $\text{SiO}_2 \text{ ha}^{-1}$ , respectively. The positive effect of foliar applications of silicon fertilizer was achieved only for soybean plants. The combined soil and foliar application of 60 kg  $\text{SiO}_2 \text{ ha}^{-1}$  and 30 kg  $\text{SiO}_2 \text{ ha}^{-1}$ , respectively, gave the maximum yield increase. The response of crops to the incorporation of potassium silicate into the soil expressed as yield increase compared to the control was in the following order: spring barley < silage corn < soybean. Based on the shown effectiveness of the use of silicon fertilizers under various soil and climatic conditions in other countries, further research is needed to establish the mechanisms of action of Si in various forms of silicon fertilizers and the methods of their application on chernozem soils in Ukraine.

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KORISNI UTICAJI SILICIJUMSKOG ĐUBRIVA NA POKAZATELJE  
KLIJANJA SEMENA, PRINOSA ZRNA JEČMA I  
SOJE I BIOMASE SILAŽNOG KUKURUZA

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R e z i m e

Cilj rada bio je da se utvrde optimalne doze i načini primene silicijumskog đubriva kod ječma, silažnog kukuruza i soje u klimatskim uslovima Ukrajine. Izvršen je niz laboratorijskih i poljskih ogleda, kao i statistička i analitička obrada podataka. Poljski ogled malih parcela je izveden na zemljištu tipa podzolasti černozem u NNC „Institut za pedološka i agrohemija istraživanja po imenu O.N. Sokolovski” (Harkovska oblast, šumo-stepe Ukrajine). Laboratorijski ogledi su postavljeni u Petrijevim posudama korišćenjem različitih koncentracija kalijum silikata (0%, 0,5%, 0,75% i 1,0%) za procenu pokazatelja klijanja semena ječma i kukuruza. Pokazano je značajno stimulativno dejstvo kalijum silikata na klijanje i energiju klijanja semena ječma i kukuruza i utvrđena optimalna koncentracija rastvora za predsetveno tretiranje semena. Utvrđena je visoka pozitivna korelacija između količine primene  $\text{SiO}_2$  i prinosa proučavanih useva ( $P<0,01$ ;  $R=0,7479-0,8682$ ). Utvrđeni su optimalni nivoi predsetvenog unošenja  $\text{SiO}_2$  u zemljište za postizanje maksimalnih prinosa na zemljištu tipa podzolasti černozem (105 kg  $\text{SiO}_2 \text{ ha}^{-1}$  za ječam, 92 kg  $\text{SiO}_2 \text{ ha}^{-1}$  za silažni kukuruz i 76 kg  $\text{SiO}_2 \text{ ha}^{-1}$  za soju). Takođe, za svaki od proučavanih useva utvrđeni su optimalni načini primene silicijumskih đubriva, koji će poboljšati njihovu produktivnost na zemljištima tipa černozem.

**Ključne reči:** silicijum, ishrana biljaka, produktivnost useva, mineralno đubrivo.

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## APPLICATION OF SODIUM SELENITE IN THE GROWING TECHNOLOGY OF WHITE LUPIN (*LUPINUS ALBUS* L.)

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**Abstract:** *Lupinus* is a very diverse genus with many species. The aim of this study was to determine the influence of different methods of application of sodium selenite on the nutritional value of white lupine variety Degas. The highest effect on the content of crude protein was obtained by spraying vegetating plants before the beginning of the flowering phase. In this variant, a class 1 grain was obtained while meeting other requirements of the feed lupin quality standard. This study reveals that the use of selenium contributed to increasing the collection of crude protein at the harvest of white lupin. It is assumed that selenium stimulated the processes of nitrogen entering plants and its redistribution from vegetative to generative organs, as well as activated the synthesis of proteins and their accumulation in the grain of white lupin. It was found that the content of alkaloids in the resulting crop of white lupin did not exceed acceptable levels, which allows it to be used for feed purposes and the preparation of various types of feed. The use of selenium contributed to the increase in the collection of crude protein content at the harvest of above-ground plant mass as it stimulates nitrogen processes in plants and its redistribution from vegetative organs to generative organs, as well as the synthesis of protein compounds in the lupin grain.

**Key words:** white lupin, sodium selenite, methods of application, spraying, crude protein content.

### Introduction

Lupin species could be an actual sustainable alternative source of protein for animal feeding. However, only four of them – namely, *L. albus*, *L. angustifolius*, *L. luteus* and *L. mutabilis* – are cultivated. *Lupinus* is a very diverse, widespread genus of the *Fabaceae* family with numerous species. It is distributed in a wide range of climatic conditions, from subarctic regions to semi-deserts and subtropical

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climates, as well as from sea level to alpine ecosystems (Jezierny et al., 2010; Annicchiarico et al., 2014; Vishnyakova et al., 2021).

Recently, there has been a significant increase of interest in improving technologies for growing white lupin in the non-black soil zone, which makes it possible to increase its distribution area and solve the problem of obtaining high-protein feed for livestock. Lupin, being a high-protein field crop, is also characterized by the content of essential amino acids, which makes it possible to obtain feeds that are qualitatively better than those of other forage crops (Tsygutkin et al., 2011; Abraham et al., 2019).

Lupins are mostly used as fodder and food crops, and some species are also used ornamenteally (Yaver and Bilgili, 2021; Arnoldi et al., 2015; Fedulova et al., 2019).

The researchers address the main proteins of white lupin seed (*Lupinus albus*, L.) and report on the current state of knowledge of the structural and functional properties of these proteins with the aim of providing the first comprehensive, accurate and up-to-date survey on this topic. Of particular interest are the molecular and biological features of the four main protein families of lupin seed globulins, called  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -conglutins. Their nutritional, technological, nutraceutical and allergenic potential is also important (Duranti et al., 2008; Shrestha et al., 2021).

At the same time, one of the disadvantages of lupin is the content of biologically active substances in the seeds and green mass – alkaloids, which negatively affect the living organism and reduce the nutritional quality of the product. Therefore, reducing the content of alkaloids in the grain and green mass of lupin improves the feed value of the resulting products (Vishnyakova et al., 2020).

This makes it possible to produce a full-fledged, environmentally safe vegetable feed protein, suitable for the preparation of concentrated and other types of feeds.

In addition, lupin has an agronomic importance (green manure), which allows increasing the stability of farming systems (Lucas et al., 2015).

The most productive of all lupin species is the white lupin (*Lupinus albus* L.) – grown in many countries and used for feed production and as a food crop. White lupin is characterized by the highest protein yield per unit area, surpassing other types of lupin (Lukashevich et al., 2018).

The aim of the research was to study various ways of applying sodium selenite to the feed value of the white Dega lupin crop.

## Material and Methods

In order to examine the various ways of applying of sodium selenite, a field micro-experiment was conducted at the experimental site of the Department of Agronomic, Biological Chemistry and Radiology of the Russian State Agrarian

University – Timiryazev Moscow Agricultural Academy within three years. The subject of this study was a white lupin of the Degas variety. Lupin is a white variety of Degas, which is a variety of universal use, bred by the Lupin Research Institute and the Russian State Agrarian University – Timiryazev Moscow Agricultural Academy. In 2004, the variety was included in the state register of selection achievements (Gataulina et al., 2013). The experiment was carried out on urbanozem soil type with following agrochemical characteristics: exchange acidity close to neutral ( $\text{pH}_{\text{HCl}}$ ) – 6.0 (GOST 26483-85), hydrolytic acidity 0.9 (GOST 26212), the amount of absorbed bases (S) – 24.3 (GOST 27821-88). The humus content was 3.3% (GOST 26213-91). The presence of mobile forms of phosphorus – 125 mg/kg of soil (class IV), potassium – 120 mg/kg of soil (class IV) (according to Kirsanov) (GOST 26207-91).

The design of the experiment: as a one-factorial using the block system in four repetitions, vegetation plot shape – square, the total area of the plot was 1  $\text{m}^2$ , the registered area of the plot was 0.64  $\text{m}^2$ . All research results were statistically processed by using the one-way analysis of variance.

Mineral nutrients were prepared by manual addition of ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), ammophos ( $\text{NH}_4\text{H}_2\text{PO}_4$ ) and single-substituted potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) at a depth of 7–10 cm. In all variants, nitrogen, phosphorus and potassium were added at the rate of N20P115K145.

Selenium was applied in two ways: pre-seed treatment (PST) by soaking the seeds and spraying the vegetating plants (SVP) before the beginning of the flowering phase and after flowering with a 0.01% solution of sodium selenite salt ( $\text{Na}_2\text{SeO}_3$ ). The control was a variant in which no sodium selenite was used.

After harvesting, the main white lupin quality indicators were determined using the SpectraStar 2500XL-R device. Sampling and preparation for analysis using the near-infrared spectroscopy method was performed in accordance with GOST 32040-2012. To determine the reliability of the obtained results, mathematical processing was employed using the multivariate method of variance analysis (Kobzarenko et al., 2015).

## Results and Discussion

The main characteristic of the nutritional value of feed crops is the assessment of the impact of growing conditions on the content of crude protein in the grain and green mass and the collection of crude protein at harvest.

As obtained in field studies conducted with white lupin, the highest content of raw protein was obtained in the grain of the crop (Figure 1). It was found that the use of sodium selenite contributed to an increase in the crude protein content in the grain of white lupin. The highest amount of crude protein was obtained by spraying plants in the vegetative phase. This value increased to 39.6% compared to the

control variant (31.4%). It was found that, when using sodium selenite, the content of crude protein in the stems and pods decreased. It is assumed that the use of sodium selenite activated the processes of redistribution of assimilates from vegetative to generative organs and stimulated the synthesis of protein compounds in the grain, which led to an increase in the crude protein content in the grain of white lupin and an increase in its nutritional value.

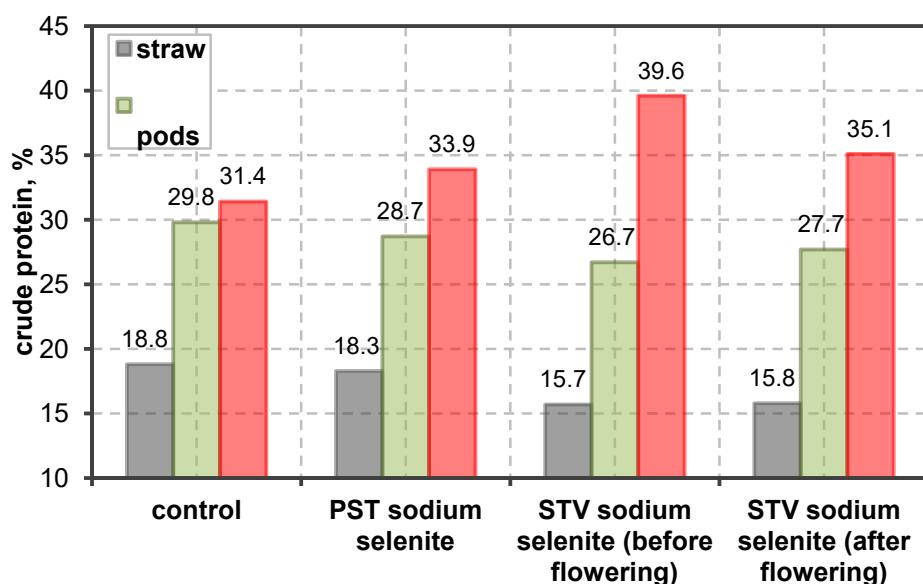


Figure 1. The crude protein content in white lupin plants when using sodium selenite.

In accordance with GOST R 54632-2011, the grain of white lupin is classified into classes 1, 2 and 3 in terms of crude protein content, subject to other requirements. The grain obtained in our study, when spraying the plants in the vegetative phase before the beginning of the flowering can be classified as class 1 in terms of crude protein content. In this variant, a grain with a crude protein content of 39.6% was obtained. When using sodium selenite by pre-sowing seed treatment and spraying the vegetating plants after the flowering phase, the grain can be classified as class 3. The crude protein content in these variants was 34.9% and 35.1%, respectively.

The results of research show that the use of sodium selenite contributed to an increase in the collection of crude protein compared to the control variant (Table 1). Most of the increase in crude protein collection was in the lupin stalks and pods. However, the contribution of each component to the total crude protein collection

by the above-ground mass of the plants differed, depending on the method of processing with sodium selenite. It was found that when using pre-sowing seed treatment, the total collection of crude protein by the above-ground mass of the white lupin crop was  $403.2 \text{ g/m}^2$ , which was 23% more than in the control variant.

At the same time, the collection of raw protein with a crop of stems has not changed, the collection of raw protein with a grain harvest has increased by 14%, the collection of raw protein with a crop of pods has more than twice.

Spraying plants with sodium selenite before the beginning of the flowering phase contributed to a significant increase in the crude protein collection in a grain and leaf harvest by more than twice.

At the same time, in this variant, the yield of crude protein in the crop of stems sharply decreased by 30%, which did not lead to a significant increase in the total yield of crude protein in the crop of aboveground mass of plants.

Spraying vegetating plants with sodium selenite after the flowering phase contributed to a 35% increase in the total collection of raw protein by the above-ground mass of plants. Also, the contribution of grain to the total collection of crude protein by above-ground plant mass increased by 1.9 times and the contribution of flaps increased by 1.6 times compared to the control variant. Thus, it can be concluded that the use of selenium contributed to an increase in the nutritional value of the resulting crop of white lupin by stimulating the nitrogen supply of plants and activating the synthesis of protein compounds.

Table 1. The collection of crude protein in a crop of Dega lupin using sodium selenite.

Variant	Method of processing	Crude protein, $\text{g/m}^2$			
		grain	pods	straw	mass of plants
control		42.3	49.2	237.0	328.5
sodium selenite	PST	48.3	116.1	238.8	403.2
sodium selenite	STV before flowering	95.1	80.1	165.0	34.2
sodium selenite	STV after flowering	78.9	149.7	203.4	432.0
SSD* <sub>05</sub>		3.3	7.0	12.5	-

\*the smallest significant difference.

One of the indicators for assessing the nutritional value of feed products, including white lupin, and suitability for feed production, is the content of alkaloids (Shakirov et al., 2016). In accordance with the existing classification of feed, the content of alkaloids in the products obtained from white lupin of all variants did not exceed the permissible amounts (Table 2). It is shown that the content of alkaloids in the grain of white lupin is 0.04–0.06%, in the pods – 0.03–0.04 %, whereas, it was not found in the stems.

Table 2. The alkaloid content in the above-ground mass of white lupin plants.

Variant	Method of processing	Alkaloid content, %		
		in the grain	in the pods	in the stems
control		0.06	0.04	-
sodium selenite	PST	0.06	0.03	-
sodium selenite	STV before flowering	0.04	0.04	-
sodium selenite	STV after flowering	0.05	0.03	-
SSD <sub>05</sub>		0.01	0.01	-

### Conclusion

The results of research have shown that the use of selenium contributes to an increase in the nutritional value of the crop of white lupin, as a result of increasing the content of crude protein in the grain and accumulation of crude protein at the harvest of above-ground plant mass. The highest crude protein content (39.6%) was obtained by spraying the plants with sodium selenite before the beginning of the flowering phase. If all the requirements of the quality standard for feed lupin are met, the grain obtained in this variant can be classified as class 1. This study reveals that the use of selenium contributed to increasing the collection of crude protein at the harvest of white lupin. It is assumed that selenium stimulated the processes of nitrogen entering plants and its redistribution from vegetative to generative organs, activated the synthesis of proteins and their accumulation in the grain of white lupin. It was determined that the content of alkaloids in the resulting crop of white lupin did not exceed acceptable levels, which allows it to be used for feed purposes and the preparation of various types of feed.

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PRIMENA NATRIJUM SELENITA U TEHNOLOGIJI GAJENJA  
BELE LUPINE (*LUPINUS ALBUS L.*)

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R e z i m e

*Lupinus* je veoma raznovrstan rod sa mnogo vrsta. Cilj ovog istraživanja bio je da se utvrdi uticaj različitih metoda primene natrijum selenita na nutritivnu vrednost bele lupine, sorte Degas. Najveći efekat na sadržaj sirovih proteina postignut je tretiranjem biljaka u vegetativnoj fazi pre početka faze cvetanja. Kod ove varijante, dobijeno je zrno klase 1 uz ispunjavanje ostalih zahteva standarda kvaliteta krmne lupine. Ova studija pokazuje da je upotreba selena doprinela povećanju nakupljanja sirovih proteina prilikom žetve bele lupine. Pretpostavlja se da je selen stimulisao procese ulaska azota u biljke i njegove preraspodele iz vegetativnih u generativne organe, kao i da je aktivirao sintezu proteina i njihovu akumulaciju u zrnu bele lupine. Utvrđeno je da sadržaj alkaloida u usevu bele lupine ne prelazi prihvatljive nivoje, što omogućava da se koristi za ishranu i pripremu raznih tipova hraniva. Upotreba selena doprinela je povećanju nakupljanja sirovih proteina prilikom žetve nadzemne mase biljaka zbog stimulisanja procesa azota u biljkama i njegove preraspodele iz vegetativnih organa u generativne organe, kao i sinteze proteinskih jedinjenja u zrnu lupine.

**Ključne reči:** bela lupina, natrijum selenit, načini primene, tretiranje, sadržaj sirovih proteina.

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PROXIMATE, MINERAL, VITAMIN, AND ANTI-NUTRIENT  
CONTENTS OF THE LEAVES OF *SENECIO BIAFRAE*

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**Abstract:** Micronutrient malnutrition is a global challenge. However, there are promising opportunities for combating it through the consumption of neglected and underutilized leafy green vegetables. *Senecio biafrae* is an underutilized and nutrient-rich green leafy vegetable with huge nutritional and health potentials that have remained unexploited. The aim of this research was to evaluate the proximate, mineral, vitamin, anti-nutrient contents of the leaves of *Senecio biafrae*. Leaves of *Senecio biafrae* were collected from five communities in Ekiti State and analyzed using standard biochemical methodologies. The results showed that the leaves differed significantly in nutritional and anti-nutritional contents. The leaves were rich in potassium, magnesium, and calcium, and low in fat and anti-nutrient contents for all the groups studied. The Pearson's correlation results showed that most of the nutritional parameters either had inverse or no relationships with anti-nutrients. The crude protein showed significantly positive correlations with dry matter (0.90\*\*) and a negative correlation with cyanogenic glycosides (-0.90\*\*). Cyanogenic glycosides showed significantly negative correlations with potassium (-0.63\*\*), calcium (-0.66\*\*) and dry matter (-0.44\*). Nitrate showed no significant relationship with any nutritional parameter. Oxalate and tannin showed no significant relationship with the vitamins. Phytate and tannin showed no significant relationship with the proximate contents and minerals. The results showed that *Senecio biafrae* leaf is nutrient-rich and could help to mitigate the effects of micronutrient deficiencies. The variations and relationships among the nutritional and anti-nutritional parameters could enhance meaningful selection and nutritional quality through breeding.

**Key words:** nutrient-dense, leafy vegetable, *Senecio biafrae*, minerals, vitamins, proximate, anti-nutrients, micronutrients.

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## Introduction

Malnutrition used to be considered a challenge of the resource-poor people alone until the problems of micronutrient (“hidden-hunger”) malnutrition became prevalent among the elites and the resource-poor. Green leafy vegetables, however, have huge underexploited potentials for mitigating the effects of hidden-hunger and combating malnutrition. In most parts of the world, the consumption of leafy vegetables is not cherished by the younger generations, especially children. Edema (1987) noted that the dislike for leafy green consumption could be attributed partly to the fact that some leafy vegetables might be bitter. Some have a peculiar aroma and some may not be so tasty. Sub-Saharan Africa, for instance, despite the vast biodiversity of green leafy vegetables, consumes them the least (Remi et al., 2005). Crop biodiversity and the nutritional contents of vegetable crops are particularly important for food and nutrition security on a global scale. Leafy greens are extremely important to one’s health and illness prevention (Hanif et al., 2006), and they are some of the cheapest and some of the most accessible sources of protein, carbohydrates, vitamins, minerals, and vital amino acids as well (Aregheore, 2012).

The middle-belt and southern parts of Nigeria, especially the tropical rainforests and the derived guinea savanna agro-ecologies of the country, have a huge biodiversity of green leafy vegetables cherished for their numerous food, nutritional and medicinal advantages. Most of these indigenous green leafy vegetables are harvested directly from the wild, with no serious cultivation or genetic improvement being carried out on them. There is currently massive genetic erosion of the biodiversity of these invaluable vegetables due to little or no conservation and breeding efforts being carried out on them that could enhance their growth, yield, and nutrient qualities. Underexploited and neglected crops are now being explored for nutrition and medicine because of the promising opportunities they offer (Baiyeri et al., 2018).

*Senecio biafrae* is one of these indigenous green leafy vegetables in the southern part of Nigeria. The consumers’ demand for this vegetable is usually by far higher than the supplies, which makes it comparably costlier than the other green leafy vegetables consumed in the region. A major reason for this is that *Senecio biafrae* is still currently harvested from the wild and no serious effort is being put by farmers to include it in their regular traditional cropping systems. In ethno-medicine, it is used in the treatment of hypertension, low-sperm count, pile, dysentery, cough, pulmonary defects and rheumatism (Bello et al., 2018). In spite of the huge nutritional and nutraceutical benefits associated with *Senecio biafrae*, there are limited research projects and little or no funding for research into the various aspects of this vegetable. There is also poor awareness of its dietary and medicinal potentials. Nutrient profiling is one of the strategies for unraveling the nutritional potentials of a crop and could enhance awareness about it. When

compared to other commonly consumed vegetables, there are few documented nutritional studies on this vegetable in the literature. The earlier nutritional studies on *Senecio biafrae* used one accession for their nutritional analyses (Ajala, 2009; Nupo et al., 2013; Adeniji, 2014). These studies looked at proximate composition and either minerals or anti-nutrients. Evaluating the nutritional and anti-nutritional qualities of a food crop could unveil the variations in its genotypes with respect to the studied traits. This is a prerequisite for the improvement of such traits through crop breeding. To the best of the writers' knowledge, there are no documented studies on the proximate, mineral, vitamin, anti-nutrient contents and their correlations using a number of accessions in *Senecio biafrae*. Evaluating the nutritional and anti-nutritional composition of crops, especially their correlations using various genotypes could provide useful information to nutritionists, agronomists and crop breeders that will enhance the designing of dietary preparations, crop management and breeding strategies, all of which will ultimately be of immense benefit to the consumers. In order to enhance sustainable food and nutritional security, nutritionists, agronomists, and crop breeders need to look beyond yield and yield-components alone and begin to take into consideration the nutritional and anti-nutritional traits and the inter-relationships among them in vegetables and staple food crops. This study was therefore initiated to evaluate the proximate, mineral, vitamin, anti-nutrient contents and their correlations in *Senecio biafrae* accessions collected in Ekiti State, southwestern Nigeria.

## Material and Methods

*Leaf collection and preparation:* The vines with leaves of *Senecio biafrae* were collected from five communities in Ekiti State: Aba oyo, Ijebu agege, Ijesha isu, Odooro, Okeorin. The accessions were named after the communities where they were collected. The samples were kept in a cooling system and transported to the laboratory. The leaves were detached from the vine and washed carefully with distilled water and drained. The fresh leaf samples were homogenized with a mortar and pestle and weighing was done for the various analyses. Proximate, mineral, vitamin, and anti-nutrient analyses were performed. All biochemical analyses were carried out in quadruplicates.

*Proximate analysis:* The assays were performed using the established techniques of analysis outlined by AOAC (2005). The crude protein concentration in the samples was determined using the standard semi-micro Kjeldahl method – AOAC (2005) method 988.05. Crude fat in the samples was analyzed using AOAC (2005) method 2003.06. Dry matter and moisture contents of the samples were determined using AOAC (2005) method 967.08. The ash concentrations were determined using AOAC (2005) method 942.05. The crude fibre was analyzed using AOAC (2005) method 958.06. The carbohydrate was determined by

difference. This was done by subtracting (% moisture + % crude protein + % ether extract + % crude fiber + % ash) from 100%.

*Mineral content determination:* The mineral contents (calcium [Ca], zinc [Zn], iron [Fe], magnesium [Mg], copper [Cu], sodium [Na]) of the leaf samples of the *Senecio biafrae* accessions were analyzed using AOAC (2005) method 975.11. The digest of each sample as obtained from sample dissolution was aspirated into the Buck 210 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combinations. Phosphorus was determined by the vanado-molybdate spectrophotometric method (AOAC, 975.16).

*Vitamin analyses:* The concentrations of vitamin A, vitamin B1, vitamin B2, vitamin B5, vitamin B6, vitamin C, vitamin E and vitamin K were assayed in the leaf samples of the *Senecio biafrae* accessions using the AOAC (2005) method.

*Anti-nutrient determination:* The phytate contents of the samples were determined using the AOAC (2006) method. Nitrate in the samples was analyzed using the rapid colorimetric method by nitrification of salicylic acid (Cataldo et al., 1975). Oxalate concentration was analyzed using the AOAC 2006 method. Tannin contents in the leaf samples were analyzed using the AOAC 2006 method. Cyanogenic glycoside was analyzed in the samples using AOAC (2005) method 915.03.

*Statistical analysis:* All data collected were analyzed using the *R* statistical analysis package, version 4.1.1. The analysis of variance was done using the library *Agricolae*. The significance of the treatment means was determined by the Tukey's HSD test at the 5% probability level, and the standard deviation was determined using the library (*Psych*). Pearson's correlation analysis was done using the library: *Hmisc* to understand the strength of the relationships among the nutritional and anti-nutritional traits studied in the *Senecio biafrae* accessions.

## Results and Discussion

The results of the proximate composition of the *Senecio biafrae* accessions are shown in Table 1. Proximate composition was significantly ( $p<0.05$ ) influenced by accession. Crude protein was significantly affected by accession and ranged from 1.76% in Ijesha isu to 2.29% in Aba oyo that recorded the highest crude protein content. The crude protein levels in these accessions were within the range reported by Nupo et al. (2013) for *Senecio biafrae*. The accessions significantly ( $p<0.05$ ) varied in their crude fat and crude fiber concentrations. Aba oyo had the highest crude fat content (1.34%) while Okeorin (1.18%) had the least fat content. The fat content was within the range reported for *Senecio biafrae*, *Solanum nigrum* and *Crassocephalum crepidioides* by Nupo et al. (2013) and comparable to the crude fat

(0.92%) reported for *Senecio biafrae* by Ajala (2009). The low fat content of these *Senecio biafrae* accessions makes them a promising leafy vegetable in the treatment of overweight and obesity. The crude fibre content was also significantly influenced by the accessions, with Aba oyo (2.52%) having the highest content; followed closely by Ijebu agege (2.40%) while Okeorin (2.25%) had the least crude fiber among the accessions. The results agree with the crude fiber content of commonly consumed fresh vegetables reported by Titchenal and Dobbs (2006). Higher crude fiber contents have been reported for *Senecio biafrae* in dried leaf samples (Famurewa, 2009; Gbadamosi and Okolosi, 2013; Nupo et al., 2013). The consumption of fiber has been associated with a reduction in serum cholesterol levels, lower risks of coronary heart disease, high blood pressure, diabetes, and colon and breast cancers (Ishida et al., 2000; Ramula and Rao, 2003). Okeorin recorded the highest value (2.16%) for the total ash content, while Aba oyo (1.65%) recorded the lowest value for the total ash content. The ash contents of the accessions analyzed were within the values (1.8–2.5%) reported for the same vegetable by Nupo et al. (2013). Aba oyo recorded the least moisture content (90.47%) and the highest dry matter content (9.53%) while Ijesha isu that recorded the highest moisture content (91.23%) also had the least dry matter content (8.76%). The moisture contents of the fresh leaf samples analyzed were equivalent to 89.68% and 89.49% reported for *Senecio biafrae* and *Solanum nigrum*, respectively (Ajala, 2009).

Table 1. Proximate composition (%) of the fresh leaves of *Senecio biafrae* accessions.

Accession	Crude protein ( $\pm$ SD)	Crude fat ( $\pm$ SD)	Crude fibre ( $\pm$ SD)	Total ash ( $\pm$ SD)	Moisture ( $\pm$ SD)	Dry matter ( $\pm$ SD)	Carbohydrate ( $\pm$ SD)
Aba oyo	2.29 $\pm$ 0.07 <sup>a</sup>	1.34 $\pm$ 0.06 <sup>a</sup>	2.52 $\pm$ 0.04 <sup>a</sup>	1.88 $\pm$ 0.03 <sup>c</sup>	90.47 $\pm$ 0.06 <sup>c</sup>	9.53 $\pm$ 0.06 <sup>a</sup>	1.41 $\pm$ 0.12 <sup>a</sup>
Ijebu agege	2.01 $\pm$ 0.35 <sup>abc</sup>	1.23 $\pm$ 0.05 <sup>b</sup>	2.40 $\pm$ 0.06 <sup>ab</sup>	2.02 $\pm$ 0.07 <sup>b</sup>	90.92 $\pm$ 0.42 <sup>ab</sup>	9.08 $\pm$ 0.42 <sup>bc</sup>	1.59 $\pm$ 0.16 <sup>a</sup>
Ijesha isu	1.76 $\pm$ 0.16 <sup>c</sup>	1.20 $\pm$ 0.11 <sup>b</sup>	2.24 $\pm$ 0.18 <sup>b</sup>	2.10 $\pm$ 0.07 <sup>ab</sup>	91.24 $\pm$ 0.06 <sup>a</sup>	8.76 $\pm$ 0.06 <sup>c</sup>	1.28 $\pm$ 0.12 <sup>b</sup>
Okeorin	1.95 $\pm$ 0.17 <sup>bc</sup>	1.18 $\pm$ 0.05 <sup>b</sup>	2.25 $\pm$ 0.13 <sup>b</sup>	2.16 $\pm$ 0.03 <sup>a</sup>	90.91 $\pm$ 0.45 <sup>ab</sup>	9.09 $\pm$ 0.45 <sup>bc</sup>	1.59 $\pm$ 0.13 <sup>a</sup>
Orinodo	2.20 $\pm$ 0.23 <sup>ab</sup>	1.23 $\pm$ 0.02 <sup>b</sup>	2.37 $\pm$ 0.07 <sup>ab</sup>	2.10 $\pm$ 0.09 <sup>ab</sup>	90.61 $\pm$ 0.10 <sup>bc</sup>	9.39 $\pm$ 0.10 <sup>ab</sup>	1.59 $\pm$ 0.07 <sup>a</sup>

SD = Standard deviation; Means followed by different letters are significantly different by a Tukey's HSD test at  $\alpha = 0.05$ .

The high moisture contents of the *Senecio biafrae* accessions suggest the need for effective postharvest handling and storage methods with minimal nutrient loss, hence preventing huge postharvest losses associated with leafy vegetables that have high moisture contents. The accessions differed significantly ( $p < 0.05$ ) in their carbohydrate contents, which ranged from 1.29 to 1.59%. All the accessions were statistically higher than Ijesha isu (1.29%) in their carbohydrate contents.

Vegetables are known for very little carbohydrate and low fat but provide concentrated sources of minerals and vitamins (Titchenal and Dobbs, 2006). The variation in the proximate composition of the *Senecio biafrae* accessions evaluated in this study suggests the possibility of improving these nutritional qualities through crop improvement. The germplasm level of diversity is critical to a successful crop improvement plan (Adebisi, et al., 2001).

*The mineral composition:* The accessions significantly ( $p<0.05$ ) differed in their iron (Fe), magnesium (Mg), phosphorus (P) contents (Table 2). Orinodo (28.19 mg/kg) recorded the highest value for iron. Okeorin (20.29 mg/kg) had the least iron concentration. The zinc (Zn) contents of the accessions ranged from Ijebu agege (3.88 mg/Kg) and Orinodo (4.01 mg/kg). Calcium (Ca) ranged from Orinodo (574.60 mg/kg) to Okeorin (618.90 mg/kg). Magnesium contents of *Senecio biafrae* ranged from Ijebu agege (425.30 mg/kg) to Orinodo (500.30 mg/kg) which was significantly higher than the rest of the accessions in their magnesium concentrations. Ijesha isu (19.92 mg/kg) was the most prominent accession for phosphorus and was significantly ( $p<0.05$ ) higher than Aba oyo (15.28 mg/kg) that recorded the least phosphorus content. The *Senecio biafrae* accessions were not significantly ( $p>0.05$ ) different in their potassium concentrations. The potassium content ranged from 409.15 mg/kg in Ijesha isu to 427.30 mg/kg in Ijebu agege. The sodium contents were also statistically similar in the accessions. Sodium ranged from 184.60 mg/kg in Aba oyo to 196.70 mg/kg in Ijesha isu. The accessions were statistically similar in their copper concentrations, with Orinodo (4.03 mg/kg) recording the highest value, while Ijebu agege had the lowest concentration of the mineral. The results obtained in this study for the minerals (Fe, Zn, Mg, K, Ca, P, Cu, and Na) were within the range of values reported for minerals in leafy vegetables (Titchenal and Dobbs, 2006; Garcia-Herrera et al., 2020).

Table 2. Mineral composition (mg/kg) of the fresh leaves of *Senecio biafrae* accessions.

Accession	Iron ( $\pm$ SD)	Zinc ( $\pm$ SD)	Minerals Calcium ( $\pm$ SD)	Magnesium ( $\pm$ SD)	Phosphorus ( $\pm$ SD)	Potassium ( $\pm$ SD)	Sodium ( $\pm$ SD)	Copper ( $\pm$ SD)
Aba oyo	20.82 $\pm$ 1.11 <sup>b</sup>	3.89 $\pm$ 0.33 <sup>a</sup>	586.70 $\pm$ 1.30 <sup>ab</sup>	446.90 $\pm$ 8.43 <sup>ab</sup>	15.28 $\pm$ 0.47 <sup>b</sup>	419.40 $\pm$ 4.36 <sup>a</sup>	184.60 $\pm$ 4.50 <sup>b</sup>	4.00 $\pm$ 1.12 <sup>a</sup>
Ijebu agege	23.42 $\pm$ 3.98 <sup>b</sup>	3.88 $\pm$ 0.86 <sup>a</sup>	588.10 $\pm$ 38.91 <sup>ab</sup>	425.30 $\pm$ 4.38 <sup>c</sup>	18.05 $\pm$ 1.48 <sup>a</sup>	427.30 $\pm$ 29.33 <sup>a</sup>	195.20 $\pm$ 8.59 <sup>ab</sup>	3.62 $\pm$ 0.38 <sup>a</sup>
Ijesha isu	28.98 $\pm$ 0.77 <sup>a</sup>	3.98 $\pm$ 0.27 <sup>a</sup>	575.40 $\pm$ 10.94 <sup>b</sup>	459.80 $\pm$ 29.27 <sup>b</sup>	19.92 $\pm$ 0.37 <sup>a</sup>	409.10 $\pm$ 10.60 <sup>a</sup>	196.70 $\pm$ 4.82 <sup>a</sup>	3.73 $\pm$ 0.41 <sup>a</sup>
Okeorin	20.29 $\pm$ 2.57 <sup>b</sup>	3.94 $\pm$ 1.09 <sup>a</sup>	618.90 $\pm$ 27.31 <sup>a</sup>	435.30 $\pm$ 26.27 <sup>ab</sup>	18.70 $\pm$ 0.23 <sup>a</sup>	416.30 $\pm$ 5.99 <sup>a</sup>	189.30 $\pm$ 10.79 <sup>ab</sup>	4.39 $\pm$ 0.72 <sup>a</sup>
Orinodo	28.19 $\pm$ 1.90 <sup>a</sup>	4.01 $\pm$ 0.34 <sup>a</sup>	574.60 $\pm$ 24.60 <sup>b</sup>	500.30 $\pm$ 2.15 <sup>a</sup>	18.76 $\pm$ 2.31 <sup>a</sup>	415.10 $\pm$ 7.07 <sup>a</sup>	185.50 $\pm$ 5.34 <sup>b</sup>	4.03 $\pm$ 0.45 <sup>a</sup>

SD = Standard deviation; Means followed by different letters are significantly different by a Tukey's HSD test at  $\alpha = 0.05$ .

**The vitamin concentrations:** The accessions were similar in their vitamin concentrations except for the concentration of vitamin C, which was significantly ( $p<0.05$ ) influenced by accession (Table 3). Vitamin A ranged from 252.20 mg/100 g in Ijesha isu to 256.70 mg/100 g in Aba oyo. Vitamin B1 ranged from 0.1253 mg/100 g in Orinodo to 0.1328 mg/100 g in Okeorin. Vitamin B2 ranged from 0.1195 mg/100 g in Okeorin to 0.1210 mg/100 g in Aba oyo. Vitamin B5 ranged from 0.2382 mg/100 g in Okeorin to 0.2442 mg/100 g in Ijesha isu. Vitamin B6 ranged from 0.0525 mg/100 g in Aba oyo to 0.0528 mg/100 g in Ijesha isu. Vitamin C concentration was significantly ( $p<0.05$ ) influenced by accession. The highest vitamin C content was found in Ijesha isu (26.35 mg/100 g), which was significantly higher than in Orinodo (23.35 mg/100 g). The vitamin E contents of the accessions ranged from 42.17 mg/100 g in Okeorin to 44.65 mg/100 g in Ijebu agege while vitamin K ranged from 17.23 mg/100 g in Aba oyo and in Ijebu agege to 18.06 mg/100 g in Orinodo. The vitamin A contents in the accessions were higher than the rest of the vitamins analyzed in the samples. A similar result was reported for vitamin A in kale, spinach and duckweed (Natesh et al., 2017). The high vitamin A contents in *Senecio biafrae* are commendable, which can be attributed to the purple pigmentation on the stems and the leaves. The results of the vitamins in this study are within the ranges reported for various commonly consumed green leafy vegetables (Titchenal and Dobbs, 2006; Ojimelukwe and Amaechi, 2019). The results suggest that *Senecio biafrae* is a promising leafy vegetable with huge nutritional potentials that could help in mitigating the effects of vitamin deficiencies.

Table 3. Vitamin composition (mg/100 g) of the fresh leaves of *Senecio biafrae* accessions.

Accession	Vitamin A ( $\pm$ SD)	Vitamin B1 ( $\pm$ SD)	Vitamin B2 ( $\pm$ SD)	Vitamin B5 ( $\pm$ SD)	Vitamin B6 ( $\pm$ SD)	Vitamin C ( $\pm$ SD)	Vitamin E ( $\pm$ SD)	Vitamin K
Aba oyo	256.70 $\pm$ 2.02 <sup>a</sup>	0.1305 $\pm$ 0.00 <sup>ab</sup>	0.1210 $\pm$ 0.00 <sup>a</sup>	0.2425 $\pm$ 0.01 <sup>a</sup>	0.0525 $\pm$ 0.00 <sup>a</sup>	25.12 $\pm$ 0.33 <sup>a</sup>	42.84 $\pm$ 2.85 <sup>ab</sup>	17.23 $\pm$ 1.26 <sup>a</sup>
Ijebu agege	254.00 $\pm$ 1.36 <sup>a</sup>	0.1313 $\pm$ 0.01 <sup>ab</sup>	0.1205 $\pm$ 0.00 <sup>a</sup>	0.2410 $\pm$ 0.00 <sup>a</sup>	0.0527 $\pm$ 0.00 <sup>a</sup>	25.82 $\pm$ 0.79 <sup>a</sup>	44.65 $\pm$ 0.35 <sup>a</sup>	17.23 $\pm$ 0.39 <sup>a</sup>
Ijesha isu	252.20 $\pm$ 6.59 <sup>a</sup>	0.1293 $\pm$ 0.00 <sup>ab</sup>	0.1200 $\pm$ 0.01 <sup>a</sup>	0.2442 $\pm$ 0.01 <sup>a</sup>	0.0528 $\pm$ 0.00 <sup>a</sup>	26.35 $\pm$ 0.86 <sup>a</sup>	43.92 $\pm$ 0.81 <sup>ab</sup>	18.24 $\pm$ 1.04 <sup>a</sup>
Okeorin	253.10 $\pm$ 3.71 <sup>a</sup>	0.1328 $\pm$ 0.00 <sup>a</sup>	0.1195 $\pm$ 0.00 <sup>a</sup>	0.2382 $\pm$ 0.00 <sup>a</sup>	0.0527 $\pm$ 0.00 <sup>a</sup>	25.83 $\pm$ 0.54 <sup>a</sup>	42.17 $\pm$ 1.45 <sup>b</sup>	17.79 $\pm$ 0.54 <sup>a</sup>
Orinodo	255.20 $\pm$ 8.50 <sup>a</sup>	0.1253 $\pm$ 0.01 <sup>b</sup>	0.1200 $\pm$ 0.00 <sup>a</sup>	0.2427 $\pm$ 0.00 <sup>a</sup>	0.0527 $\pm$ 0.00 <sup>a</sup>	23.35 $\pm$ 1.78 <sup>b</sup>	42.51 $\pm$ 0.57 <sup>ab</sup>	18.06 $\pm$ 0.53 <sup>a</sup>

SD = Standard deviation; Means followed by different letters are significantly different by a Tukey's HSD test at  $\alpha = 0.05$ .

**Anti-nutrient composition:** The results of the analysis of variance for the anti-nutrients are found in Table 4. Accessions significantly ( $p<0.05$ ) affected the anti-nutrient concentrations in the leaf samples except for the phytate and tannin concentrations that were statistically ( $p>0.05$ ) similar. The accessions were

generally low in anti-nutrients. The phytate concentration of the accessions ranged from 0.3410% in Ijebu agege to 0.3682% in Okeorin. This result was within the range of 0.1–6% phytate concentration found in food items (Mohammed et al., 2002). The oxalate concentrations were significantly influenced by accession and ranged from 0.2243% in Aba oyo to 0.2495% in Orinodo and were lower than the oxalate concentrations of some commonly consumed leafy green vegetables (Badifu, 2001). Tannin concentrations of the accessions ranged from 0.0052% in Ijebu agege to 0.0058% in both Orinodo and Ijesha isu. The tannin contents of the *Senecio biafrae* accessions were also lower than 7.5% reported in *Amaranthus viridis* (Umar et al., 2011). The accessions differed significantly ( $p<0.05$ ) in their nitrate contents. Ijesha isu (0.1385%) had the highest concentration of nitrate while Ijebu agege (0.1265%) had the least nitrate concentration. The nitrate concentration is lower than 0.33% reported for *Senecio biafrae* (Ajala, 2009). The *Senecio biafrae* accessions significantly varied with respect to cyanogenic glycoside. Ijesha isu (51.63 mg/kg) had the highest concentration of cyanogenic glycoside while Okeorin (48.23mg/kg) had the least concentration of cyanogenic glycosides; these concentrations were lower than 52 mg/100 g reported for cassava leaves and 95.5 mg/100 g reported for *Vernonia amygdalina* (Aregheore, 2012). The levels of the anti-nutrients in this study were lower than the levels reported for phytate, oxalate, nitrate and tannin in the fresh leaf samples of *Senecio biafrae* and *Solanum nigrum* (Ajala, 2009). The anti-nutrients in leafy vegetables like *Senecio biafrae* pose little or no threats to humans that consume them since different food processing methods are capable of reducing them to levels that are non-toxic and facilitate the absorption of other nutrients they would have formed complexes with, thus enhancing their bioavailability. Cooking, drying and boiling have also been reported to reduce anti-nutrients in the leaves to the non-toxic level with appreciable nutrient retention in *Senecio biafrae*, *Solanum nigrum* and *Vernonia amygdalina* (Fasuyi, 2005; Ajala, 2009; Aregheore, 2012).

Table 4. Anti-nutrient composition (%) of the fresh leaves of *Senecio biafrae* accessions.

Accession	Phytate ( $\pm$ SD)	Oxalate ( $\pm$ SD)	Nitrate ( $\pm$ SD)	Tannin ( $\pm$ SD)	Cyanogenic glycosides (mg/kg) ( $\pm$ SD)
Aba oyo	0.3505 $\pm$ 0.00 <sup>a</sup>	0.2243 $\pm$ 0.01 <sup>c</sup>	0.1378 $\pm$ 0.00 <sup>a</sup>	0.0056 $\pm$ 0.00 <sup>a</sup>	48.57 $\pm$ 0.35 <sup>bc</sup>
Ijebu agege	0.3410 $\pm$ 0.05 <sup>a</sup>	0.2345 $\pm$ 0.01 <sup>bc</sup>	0.1265 $\pm$ 0.00 <sup>b</sup>	0.0052 $\pm$ 0.00 <sup>a</sup>	49.96 $\pm$ 1.65 <sup>ab</sup>
Ijesha isu	0.3638 $\pm$ 0.00 <sup>a</sup>	0.2445 $\pm$ 0.01 <sup>ab</sup>	0.1385 $\pm$ 0.01 <sup>a</sup>	0.0058 $\pm$ 0.00 <sup>a</sup>	51.63 $\pm$ 1.48 <sup>a</sup>
Okeorin	0.3682 $\pm$ 0.00 <sup>a</sup>	0.2462 $\pm$ 0.01 <sup>ab</sup>	0.1333 $\pm$ 0.00 <sup>ab</sup>	0.0055 $\pm$ 0.00 <sup>a</sup>	48.20 $\pm$ 0.93 <sup>c</sup>
Orinodo	0.3568 $\pm$ 0.01 <sup>a</sup>	0.2495 $\pm$ 0.01 <sup>a</sup>	0.1378 $\pm$ 0.01 <sup>a</sup>	0.0058 $\pm$ 0.00 <sup>a</sup>	50.36 $\pm$ 0.60 <sup>a</sup>

SD = Standard deviation; Means followed by different letters are significantly different by a Tukey's HSD test at  $\alpha = 0.05$ .

*Proximate and anti-nutrient correlations:* Crude protein showed significantly ( $p<0.01$ ) positive relationships (0.90\*\*) with dry matter and a significantly negative relationship with moisture (-0.90\*\*) and cyanogenic glycosides (-0.57\*\*). Crude fat had a negatively significant relationship with total ash (-0.63\*\*) and (-0.47\*) oxalate. Crude fat showed significantly ( $p<0.01$ ) positive relationships (0.83\*\*) with crude fiber. Oxalate showed a significantly negative relationship with crude fibre (-0.45\*). Total ash showed a significant positive relationship with oxalate (0.77\*\*). Dry matter had a significantly negative correlation (-0.44\*) with cyanogenic glycosides. Phytate, tannin and nitrate showed no relationship with proximate composition of the *Senecio biafrae* accessions. These results imply that the higher the protein, the lower the cyanogenic glycosides and the higher dry matter. Selecting or breeding for high dry matter implies increased protein and decreased cyanogenic glycosides in *Senecio biafrae*. Agronomic management practices that increase dry matter will decrease the cyanogenic glycosides and increase protein in this leafy vegetable. The inverse relationships between oxalate and crude fiber, and oxalate and fat imply that any crop management practices that enhance crude fiber and/or fat contents in this crop will decrease its oxalate concentration. The strong significant ( $p<0.01$ ) and positive correlation between total ash and oxalate implies that an increase in the total ash in this vegetable simultaneously increases oxalate content. This information should guide the vegetable breeder who would want to improve the nutrient quality of *Senecio biafrae* to select or breed for a nutrient-rich and low anti-nutritional composition of this leafy vegetable. These correlations would also give useful insights to the agronomist for enhanced crop management for optimum nutrient-density. The correlation coefficient could guide food nutritionists and scientists in the processing of this leafy vegetable for enhanced bioavailability of nutrients and better human nutrition and health. (Table 5.).

Table 5. Correlation coefficients among proximate qualities and anti-nutrients in the leaves of *Senecio biafrae* accessions.

	CP	C. fat	C. fibre	T. ash	Moist	Dry M	Phy	Tan	Oxa	Nit	C. gl
CP	1										
C. fat	0.19	1									
C. fiber	0.29	0.83**	1								
T. ash	-0.58**	-0.63**	-0.58**	1							
Moist	-0.90**	-0.34	-0.50*	0.51 *	1						
Dry M	0.90**	0.34	0.50*	-0.51*	-1.00**	1					
Phy	0.07	-0.20	-0.32	0.08	-0.06	0.06	1				
Tan	0.11	0.00	-0.36	0.04	0.04	-0.04	0.10	1			
Oxa	-0.30	-0.47*	-0.45*	0.77**	0.32	-0.32	0.13	0.01	1		
Nit	0.05	0.34	0.14	-0.13	0.06	0.04	0.23	0.45*	-0.18	1	
C. gl	-0.57**	0.09	0.12	0.23	-0.44*	-0.44*	-0.17	-0.09	0.29	0.20	1

\*A correlation is significant at the 0.05 level; \*\*A correlation is significant at the 0.01 level, CP = crude protein; C. fat = crude fat; C. fibre = crude fibre; T. ash = total ash; Moist = moisture; Dry M = dry matter; Phy = phytate; Tan = tannin; Oxa = oxalate; Nit = nitrate; C. gl = cyanogenic glycoside.

*Minerals and anti-nutrient correlations:* Cyanogenic glycosides showed significant ( $p<0.01$ ) negative correlations with calcium (-0.66\*\*), potassium (-0.63\*\*) and sodium (0.66\*\*), and a positive and significant correlation ( $p<0.05$ ) with iron (0.54\*). Tannin showed a significant ( $p<0.01$ ) negative correlation with sodium (-0.60\*\*). Oxalate showed significant ( $p<0.05$ ) positive relationships with magnesium (0.53\*). These results imply that any agronomic practices that decrease cyanogenic glycosides in *Senecio biafrae* could result in high calcium and potassium contents. This information is important considering the fact that *Senecio biafrae* is rich in calcium and potassium. The positive relationship between cyanogenic glycosides and iron gives useful information that could guide future *Senecio biafrae* breeding programs to select/breed for low cyanogenic glycosides and high minerals. Interestingly, tannin, phytate and nitrate showed no relationship with minerals in the *Senecio biafrae* accessions. (Table 6.).

Table 6. Correlation coefficients among minerals and anti-nutrients in the leaves of *Senecio biafrae* accessions.

	Fe	Zn	Ca	Mg	P	K	Na	Cu	Phy	Tan	Oxa	Nit	C. gl
Fe	1												
Zn	-0.29	1											
Ca	-0.15	-0.66**	1										
Mg	0.65**	-0.13	-0.11	1									
P	0.45*	0.25	-0.33	0.17	1								
K	0.06	-0.43	0.65**	-0.10	-0.51*	1							
Na	0.20	-0.10	-0.19	-0.12	-0.57**	-0.52*	1						
Cu	-0.27	-0.30	0.27	0.12	-0.09	-0.28	-0.07	1					
Phy	0.20	-0.23	0.30	0.12	0.00	0.16	-0.20	0.00	1				
Tan	0.09	0.03	0.00	0.19	-0.10	0.07	-0.60**	0.40	0.10	1			
Oxa	0.27	0.20	-0.07	0.53*	0.61**	-0.42	0.29	0.24	0.13	0.01	1		
Nit	0.28	-0.10	-0.22	0.21	0.06	-0.30	-0.19	0.04	0.23	0.45	-0.18	1	
C. gl	0.54*	0.07	-0.66**	0.21	0.60**	-0.63**	0.66**	-0.07	-0.17	-0.09	0.29	0.20	1

\*A correlation is significant at the 0.05 level; \*\*A correlation is significant at the 0.01 level; Fe = iron; Zn = zinc; Ca = calcium; Mg = magnesium; P = phosphorus; K = potassium; Na = sodium; Cu = copper; Phy = phytate; Tan = tannin; Oxa = oxalate; Nit = nitrate; C. gl = cyanogenic glycoside.

*Vitamin and anti-nutrient correlations:* Phytate showed a significantly ( $p<0.05$ ) negative correlation with vitamin B1 (-0.45\*). This implies that breeding efforts that improve the vitamin B1 content in *Senecio biafrae* will simultaneously reduce its phytate content. Cyanogenic glycosides showed a positive relationship with vitamin B5 (0.47\*). Breeding efforts and selection for enhanced nutritional quality in *Senecio biafrae* should explore the possibilities of identifying *Senecio biafrae* genotypes with reduced cyanogenic glycoside content and increased vitamin B5 content. Oxalate, nitrate and tannin showed no relationship with vitamins in the *Senecio biafrae* accessions. This suggests that breeding efforts or crop management practices that reduce oxalate, nitrate and tannin will not negatively affect the vitamin content of this vegetable. (Table 7.).

Table 7. Correlation coefficients among vitamins and anti-nutrients in the leaves of *Senecio biafrae* accessions.

	VA	VB1	VB2	VB5	VB6	VC	VE	VK	Phy	Tan	Oxa	Nit	C. gl
VA	1												
VB1	0.05	1											
VB2	0.15	-0.39	1										
VB5	-0.21	0.04	-0.61**	1									
VB6	0.09	-0.08	0.49*	-0.38	1								
VC	-0.41	0.59**	-0.23	0.08	-0.02	1							
VE	-0.06	-0.05	0.37	-0.45*	0.26	0.25	1						
VK	-0.18	0.18	-0.77**	0.57**	-0.51*	0.09	-0.46*	1					
Phy	0.00	-0.45*	0.00	-0.10	0.00	-0.04	-0.12	0.10	1				
Tan	-0.01	-0.24	0.35	-0.40	0.04	-0.10	0.46	-0.23	0.10	1			
Oxa	0.22	-0.09	0.11	-0.20	0.30	-0.33	-0.20	0.25	0.13	0.01	1		
Nit	-0.39	-0.25	-0.16	0.15	-0.12	0.05	-0.13	0.28	0.23	0.45*	-0.18	1	
C. gl	-0.30	-0.12	-0.24	0.47*	0.19	0.04	0.14	0.34	-0.17	-0.09	0.29	0.20	1

\*A correlation is significant at the 0.05 level; \*\*A correlation is significant at the 0.01 level; VA = vitamin A; VB1 = vitamin B1; VB2 = vitamin B2; VB5 = vitamin B5; VB6 = vitamin B6; VC = vitamin C; VE = vitamin E; VK = vitamin K; Phy = phytate; Tan = tannin; Oxa = oxalate; Nit = nitrate; C. gl = cyanogenic glycosides.

## Conclusion

The results of this study have revealed that *Senecio biafrae* is a nutrient-rich vegetable with huge potentials for combating malnutrition, especially micronutrient deficiencies and associated health challenges. The high content of vitamins A and E, potassium, magnesium, calcium, low fat and low anti-nutrient concentrations of this leafy vegetable provide some of the basis, and support the claims of the South-western people of Nigeria that greatly cherish it for its nutritional and nutraceutical advantages. The correlation results suggest the possibility to increase nutritional traits in *Senecio biafrae* without necessarily increasing most of the anti-nutrients analyzed in this study. Efforts should therefore be made to domesticate *Senecio biafrae* and include it in traditional cropping systems.

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NEPOSREDNI MINARALNI, VITAMINSKI I ANTINUTRITIVNI SADRŽAJ  
LISTOVA BILJKE *SENECIO BIAFRAE*

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R e z i m e

Neuhranjenost usled nedostatka mikronutrijenata je globalni izazov. Međutim, postoje obećavajuće mogućnosti za borbu protiv neuhranjenosti kroz konzumiranje zanemarenog i nedovoljno iskorišćenog lisnatog zelenog povrća. *Senecio biafrae* je nedovoljno iskorišćeno i hranljivim sastojcima bogato zeleno lisnato povrće sa ogromnim nutritivnim i zdravstvenim potencijalima koji su ostali neiskorišćeni. Cilj ovog istraživanja bio je da se procene neposredni mineralni, vitaminski, antinutritivni sadržaji listova biljke *Senecio biafrae*. Listovi biljke *Senecio biafrae* prikupljeni su iz pet zajednica u državi Ekiti i analizirani korišćenjem standardnih biohemijskih metodologija. Rezultati su pokazali da se listovi značajno razlikuju u pogledu nutritivnih i antinutritivnih sastojaka. Pokazalo se da su bogati kalijumom, magnezijumom i kalcijumom, i malim sadržajem masti i antinutritivnih sastojaka za sve ispitivane grupe. Rezultati Pirsonove korelacije pokazali su da većina nutritivnih parametara ima obrnutu ili nikakvu vezu sa antinutritivnim sastojcima. Sirovi proteini su bili u značajno pozitivnoj korelaciji sa suvom materijom (0,90\*\*) i u negativnoj korelaciji sa cijanogenim glikozidima (-0,90\*\*). Cijanogeni glikozidi su pokazali značajno negativne korelacije sa kalijumom (-0,63\*\*), kalcijumom (-0,66\*\*) i suvom materijom (-0,44\*). Nitrat nije pokazao značajnu vezu ni sa jednim nutritivnim parametrom. Oksalati i tanini nisu pokazali značajnu vezu sa vitaminima. Fitati i tanini nisu pokazali značajnu vezu sa mineralima. Rezultati su pokazali da je list biljke *Senecio biafrae* bogat hranljivim materijama i da može pomoći u ublažavanju efekata nedostataka mikronutrijenata. Varijacije i odnosi između nutritivnih i antinutritivnih parametara mogu poboljšati selekciju i nutritivni kvalitet kroz oplemenjivanje.

**Ključne reči:** bogat nutrijentima, lisnato povrće, *Senecio biafrae*, minerali, vitamini, neposredan, antinutrijenti, mikronutrijenti.

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COMPARATIVE EVALUATION OF THE PRODUCTIVITY OF WINTER  
CROPS (WHEAT [*TRITICUM AESTIVUM L.*], RYE [*SECALE CEREALE L.*],  
TRITICALE [*TRITICOSECALE WITT.*]) IN THE WESTERN  
FOREST-STEPPE OF UKRAINE

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**Abstract:** The aim of the study was to determine the parameters of adaptability of winter crop varieties for cultivation in the western forest-steppe zone of Ukraine (2019–2021). The subject of the study were wheat (*Triticum aestivum L.*) varieties: Vodogray bilotserkivsky, Oberig Myronivsky, Mudrist odeska; rye (*Secale cereale L.*): Knyazhe, Kobza, Kharkivivanka; triticale (*Triticosecale Witt.*): Markian, Molfar, Obrij myronivsky. The tests were carried out in the field. The area of the experimental plot was 55 m<sup>2</sup>, and the placement of varieties was consecutive with three repetitions. The results showed that under the basic cultivation technologies and weather conditions of the growing seasons, the grain productivity of soft wheat varieties varied from 4.88 (Mudrist odeska) to 5.39 t ha<sup>-1</sup> (Oberig Myronivsky); rye – from 5.55 (Kharkivivanka) to 6.32 t ha<sup>-1</sup> (Kobza); triticale – from 6.45 (Markian) to 6.74 t ha<sup>-1</sup> (Obrij myronivsky). The results obtained allow to conclude that ecologically plastic varieties of winter crops in the conditions of the studied soil-climatic zone accumulate a sufficient amount of sugar in the tillering nodes – 25–30%, ensure overwintering of plants – 94–96%, are resistant to the main diseases, which contributes to the production of high-quality seeds to ensure the cultivated area in the region and the efficiency of seed production.

**Key words:** disease, soft wheat (winter), rye (winter), triticale (winter), weight of 1000 grains, yield.

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## Introduction

Climate change and agriculture are two interconnected processes at the global level that affect the temperature regime, rainfall, atmospheric carbon dioxide and ozone concentrations, the emergence of new pests and diseases, and food quality (Deryng et al., 2011). According to the materials of the National Academy of Agrarian Sciences of Ukraine, the boundaries of the country's natural and climatic zones have actually shifted northward by 100–150 km in recent decades (Ivanyuta et al., 2020; Kovalchuk, 2019). These changes affect the grain market of Ukraine, the state of which determines the security of the country, the results of the economic activity of agricultural producers, and, in general, the well-being of the Ukrainian population (Dibrova et al., 2019; Rahman and Babenko, 2020; Kazak and Grishchenko, 2016; Kovaleva, 2015).

A science-based strategy for the development of the grain industry provides ample opportunities for effective control of the volume of grain production – the main resource potential. To support the high competitiveness of national agrarian interests, it is necessary to reliably predict the situation while maintaining the ecological balance. To achieve this, the area under cereals in Ukraine should remain relatively stable – 15–16 million hectares: of which in the steppe – about 7.1, forest-steppe – 5.6, in polissya – 2.3 million hectares. The cultivated area is expected to be distributed as follows: winter wheat – at least 6.0 million ha, winter barley – 1.2, winter rye – 0.3, corn – 5.0, spring barley – 1.5, soybeans – 2.0, millet, sorghum and buckwheat – 0.3 million ha each (Cherchel and Shevchenko, 2020).

Regional manifestations of climate change are reflected in an increase in the sum of effective temperatures and sufficient precipitation in the western forest-steppe zone, which allows the cultivation of not only cereals but also niche crops (corn, soybeans, sunflower) (Voloshchuk, 2018; Mazur et al., 2020; Yasnolob et al., 2018; Budyak and Budyak, 2020). These crops are resistant to biotic and abiotic stresses and are characterized by high yield, protein and lysine content in the grain (Petrichenko and Korniychuk, 2018; Yegupova and Romanyuk, 2020; Ryzhuk et al., 2021; Gritsenko, 2020; Wilde et al., 2018).

Litvinenko (2016) argues that the genetic yield potential of modern winter wheat varieties has increased to 8.0–10.2 t ha<sup>-1</sup> over the past decade, but is realized in production at an average of 2.62 t ha<sup>-1</sup> (25–30%).

Based on the natural and economic conditions of the main zones and the different degrees of specialization of cooperative and farm enterprises, it is advisable to use a wide range of winter crops in the structure of cultivated areas. It is possible to achieve 15–20% higher productivity of the grain field only with a scientifically based varietal policy of each farm, growing three or four varieties of

different development types (Gavrilyuk and Kalenich, 2017; Lifenko et al., 2021; Gamayunova and Litovchenko, 2017).

The western forest-steppe covers a number of subzones (forest-steppe, polesia, foothills and mountains) with different soil types characterized by low natural fertility, high acidity, leaching, and large amounts of precipitation during the formation period – grain harvesting. In this zone, selection does not take place in most winter crops, so grain producers buy the seeds of new varieties in the institutions of origin of the central forest-steppe, and even the steppe. Under these conditions, a large number of varieties of foreign selection are found in the industrial crops of the region. The modernization of the seed industry of these economically developed countries (Germany, Italy, France, Poland, Spain, Hungary, Great Britain, Romania, Greece, Portugal), whose companies operate on our territory, allows the production of high-quality seeds, which, due to calibration, encrustation with microelements, etc., underestimate the performance of domestic varieties.

The aim of the study was to determine the parameters of adaptability and productivity of winter crop varieties: soft wheat, rye, triticale for cultivation in the western forest-steppe zone of Ukraine.

## Material and Methods

The experiment was laid in the crop rotation of the Department of Seed Growing and Seed Science of the Institute of Agriculture of Carpathian region of National Academy of Agrarian Sciences (NAAS) of Ukraine in 2019–2021 (49°47'07" N, 23°52'07"E; 314 m a.m.s.l.).

The yield of varieties of three winter crops from different institutions of the owners of Ukraine was tested.

The soil of the experimental plots is a gray forest soil, superficially gleyed, light loamy, characterized by the following indicators: humus content (according to Tyurin) – 1.7%, the amount of absorbed bases – 13.7 meq per 100 g of soil, alkaline available nitrogen (according to Kornfield) – 89.6 mg kg<sup>-1</sup> of soil, phosphorus and potassium (according to Kirsanov), 69.5 and 68.0 mg kg<sup>-1</sup> of soil, respectively. Beyond the gradation, such a soil has a very low supply of nitrogen, a medium supply of phosphorus, and a low supply of potassium. The reaction of the soil solution (pH salt – 5.4) is slightly acidic.

The agrotechnology of cultivation is generally accepted for crops in this zone. The predecessor was winter rapeseed. The seeding rates for winter crops were as follows: 5.5 million viable seeds ha<sup>-1</sup> – wheat, 5.0 million viable seeds ha<sup>-1</sup> – rye, 4.5 million viable seeds ha<sup>-1</sup> – triticale. The sowing time was optimal (20–25.09 – rye, triticale; 25.09–01.10 – wheat). Seed protection included Vitavax 200 FF, 3 l t<sup>-1</sup>; plants – herbicides: roundup, 4.0 l ha<sup>-1</sup>; granstar, 75% aqueous

solution, 0.025 kg ha<sup>-1</sup>; fungicide: falcon Dou, emulsion concentrate, 0.6 l ha<sup>-1</sup>. Mineral fertilizers were applied before sowing, and post-fertilization was carried out at the stages BBCH 20-22 and BBCH 30-32. The application rate for wheat and triticale was N<sub>120</sub>P<sub>90</sub>K<sub>90</sub>, and for rye – N<sub>60</sub>P<sub>90</sub>K<sub>90</sub>. The total area of the sowing plot was 60 m<sup>2</sup> and the accounting area was 50 m<sup>2</sup>. The experiment was repeated three times.

The studies were carried out according to the generally accepted methods indicated below.

According to the data from the Lviv hydrogeological and reclamation station, the sum of the temperature regime (°C) and the amount of precipitation (mm) in the fall and winter periods and the duration of fall vegetation and dormancy of plants were determined.

For each variety, the seeding rate (R) was determined according to the following formula:

$$R = \frac{NxW \times 100}{Es} \quad (1)$$

N – number of millions of germinated seeds per 1 ha;

W – weight of 1000 seeds;

Es – economic suitability of the seed.

Economic suitability of the seed (Es) determined according to the following formula:

$$Es = \frac{\text{seed germination} \times \text{seed purity}}{100} \quad (2)$$

The germination of seeds was determined using the method of accounting plots based on the following calculations: the beginning of seedlings development (at least 15% of seedlings appeared), mass seedlings (50%), full seedlings (75% or more), the end of the phase – the emergence of the last seedlings and calculated (in percent) as the ratio of the number of seedlings to the total number of similar seeds sown (Izhik, 1976).

According to the method of Fursova et al. (2004), phenological observations of plant development during the growing season were carried out. The heading phase was determined when the inflorescence emerged at least halfway from the axil of the upper leaf, and the flowering phase was determined by the presence of anthers or stigmas. Grain maturity was determined by the organoleptic method 12–15 days after the phase of full flowering of the plants and repeated every three days. Sampling was carried out manually at 5 evenly spaced locations of each experimental plot in three incompatible replicates, then the average value of the indicator was determined. Technical maturity and harvest maturity were determined by the gravimetric drying method, which allows the timing of the onset of these stages to be determined with an accuracy of up to one day. Grain moisture above 20% was determined by oven drying and mathematically converted to the standard value of 14%.

The weight of 1000 seeds (M) was determined by 2 weighings of 500 pieces, and the average weight was calculated with an accuracy of 0.1 g. If the weight of 2 samples differed from the average by more than 0.5%, the third sample was weighed and calculated according to the following formula:

$$M = \frac{M_1 \times (100-h)}{100-Sh} \quad (3)$$

M<sub>1</sub> – mass of 1000 grains, g;

h – grain moisture, %;

Sh – standard grain moisture, 14%.

Total mono- and disaccharide content was determined by photometry using picric acid (Major et al., 2007). Sugars were extracted from samples ground in a mortar and dried in an oven (105°C) with water in a water bath (10 min at 100°C). The concentration of total sugars after acid hydrolysis (3.3% HCl) was determined colorimetrically at 490 nm using a calibration curve constructed using the scale of standard solutions of glucose or hydrolyzed sucrose. The content of dry matter in the plant material was calculated by the weight method.

Overwintering of plants of the varieties was based on the data of fall and spring registration of the state of crops in each replicate in relation to the plants that restored spring vegetation for field germination of seeds (Eshchenko et al., 2014).

The range of variation (R) in the traits of the varieties and weather conditions of the study years was determined by the difference between the maximum and minimum values.

Phytopathological evaluation was performed according to the method of Omelyuta et al. (1986) using the formula:

$$I = \frac{\sum(a \cdot b) \cdot 100}{K \cdot B} \quad (4)$$

I – disease development (in %);

Σ (a x b) – the sum of the product obtained by multiplying the number of leaves by the corresponding score;

K – total number of counted leaves (healthy and damaged);

B – the highest score on the accounting scale.

The assimilation coefficient (AC) was determined by the method of Peterson et al. (1993) using the following formula:

$$AC = (m_2 - m_1) (\ln S_2 - \ln S_1) / (S_2 - S_1) \quad (5)$$

m<sub>1</sub> i m<sub>2</sub> – dry mass of the harvest sample at the beginning and end of the accounting period, g;

ln S<sub>1</sub> i ln S<sub>2</sub> – natural logarithms of leaf areas for the accounting period;

t – duration of the experiment, days.

Biomass gain (G) per day (g m<sup>-2</sup>) was determined by the following formula:

$$G = AC \times A/a \quad (6)$$

a – number of plants per 1 m<sup>2</sup> of sowing, pcs.;

A – number of experimental plants, pcs..

Grain yield was determined by threshing with a Sampo-130 combine for each plot and weighing. Processing and summarizing of the experimental results were carried out on the computer using the Microsoft Excel program. According to the method of Ushkarenko et al. (2013), the analysis of variance was carried out according to one- and three-factor types.

## Results and Discussion

During the growing seasons of winter cereals, different weather conditions prevailed, which allowed a comprehensive evaluation of the adaptive and productive features of the varieties.

Depending on the seeding rates of a crop and variety, plant density per unit area varied within 501.0–511.4 pieces  $m^2$  for winter wheat, for rye – 458.0–460.8, for triticale – 433.6–438.0 pieces  $m^2$  (Table 1). Since the fall vegetation of the plants was longer, the varieties accumulated a sufficient amount of soluble sugars (26.9–27.1%).

Table 1. Changes in growth and sugar content in the tillering nodes of winter cereal plants at the time of fall vegetation termination (2018–2020).

Variety	Plant density, pcs $m^2$	Sugar content in tillering nodes, %	Plant height, cm	Absolute dry weight of 100 plants, g	Root system length, cm
<b>Soft wheat (winter)</b>					
Vodogray bilotserkivsky (control)	504.2	26.8	15.0	5.78	5.9
Oberig Myronivsky	511.4	27.3	15.7	6.69	6.3
Mudrist odeska	501.0	26.5	14.8	6.30	5.9
Average	505.5	26.9	15.2	6.26	6.0
SSD <sub>0.05</sub>	5.0	1.0	0.4	0.30	0.5
<b>Rye (winter)</b>					
Knyazhe (control)	460.8	26.6	18.0	8.14	7.9
Kobza	460.6	27.5	17.2	7.89	8.1
Kharkivyanka	458.0	26.8	15.8	7.63	7.4
Average	459.8	27.0	17.0	7.89	7.8
SSD <sub>0.05</sub>	3.0	0.6	1.1	0.25	0.4
<b>Triticale (winter)</b>					
Markian (control)	433.6	27.8	17.3	7.87	7.4
Molfar	438.0	27.1	17.5	8.52	7.8
Obrij myronivsky	433.6	26.3	16.3	8.44	7.3
Average	435.1	27.1	17.0	8.28	7.5
SSD <sub>0.05</sub>	2.0	0.5	0.8	0.28	0.3

The good development of the plants entering winter, the accumulation of a sufficient amount of sugars in the tillering nodes, and the abnormally warm weather conditions during the winter months led to a high percentage of

overwintering of plants of all crops. The average indicator for winter wheat was – 96.6%, for rye – 94.9, for triticale – 94.2% (Table 2). This confirms that the varieties of the different ecological types of wheat, rye and triticale are well adapted to the growing conditions of the western forest-steppe zone.

According to the mathematical processing of the data, the influences of the factors were as follows: weather conditions of the year (A) – 39%, crops (B) – 22, varieties (C) – 25, interaction of year and crop (AB) – 3, year and variety (AC) – 4, crop and variety (BC) – 2, year, crop and variety (ABC) – 2, others – 3%.

Table 2. Overwintering of plants of winter crop varieties (2019–2021), %.

Variety	Year			Average	± to control
	2019	2020	2021		
<b>Soft wheat (winter)</b>					
Vodogray bilotserkivsky (control)	96.2	95.0	99.0	96.7	-
Oberig Myronivsky	97.0	95.5	98.8	97.1	0.4
Mudrist odeska	95.8	95.2	97.6	96.2	-0.5
Average	96.3	95.1	98.8	96.6	
<b>Rye (winter)</b>					
Knyazhe (control)	95.4	88.4	99.0	94.3	-
Kobza	96.6	89.1	98.8	94.8	0.5
Kharkivyanka	94.2	87.7	98.7	95.5	-1.2
Average	95.4	88.4	98.8	94.9	
<b>Triticale (winter)</b>					
Markian (control)	94.1	89.2	98.1	93.8	-
Molfar	93.5	90.0	98.8	94.1	0.3
Obrij myronivsky	94.9	90.6	98.5	94.7	0.9
Average	94.2	89.9	98.5	94.2	
Factor:	Impact intensity			SSD <sub>0.05</sub>	
A (year)	0.39			0.93	
B (crop)	0.22			0.72	
C (grade)	0.25			0.72	
Interaction of factors:					
AB	0.03			1.61	
AC	0.04			1.61	
BC	0.02			1.25	
ABC	0.02			2.79	
Other factors	0.03				

An important indicator for determining the likely productivity of varieties is their assessment based on the photosynthetic chlorophyll potential of the leaves. In our experiments, the winter crop varieties formed a well-developed photosynthetic apparatus that was optimal in terms of volume and functional dynamics, which affected the final result – yield.

According to the dynamics of determining the total area of the leaf surface of the plants presented in Table 3, it can be seen that it increased from the second

macrostage BBCH 28–29 to the third BBCH 37–39 and was the highest. In winter wheat varieties, this indicator varied from 39.5 thousand  $m^2 \text{ ha}^{-1}$  (Mudrist odeska) to 61.5 thousand  $m^2 \text{ ha}^{-1}$  (Oberig Myronivsky). When rye was sown, the largest leaf area was recorded in the Knyazhe variety (64.8 thousand  $m^2 \text{ ha}^{-1}$ ), and in the Molfar triticale variety (70.2 thousand  $m^2 \text{ ha}^{-1}$ ). As a result of leaf death in the seventh microstage BBCH 71–75, this indicator decreased in all crops and varieties. The average assimilation coefficient at stages 3–7 BBCH 39–75 was  $-10.2 \text{ g m}^3$  of dry matter per day for winter wheat,  $12.9 \text{ g m}^3$  for rye, and  $14.5 \text{ g m}^3$  of dry matter per day for triticale.

Table 3. Total leaf area and crop assimilation coefficient (2019–2021).

Variety	Total leaf area, thousand $m^2 \text{ ha}^{-1}$			Assimilation coefficient, $\text{g m}^3 \text{ of dry matter per day}$	
	stages, code BBCH organogenesis				
	2, BBCH 28–29	3, BBCH 37–39	7, BBCH 71–75		
<b>Soft wheat (winter)</b>					
Vodogray bilotserkivsky (control)	35.7	56.7	21.5	5.7	
Oberig Myronivsky	38.1	61.5	23.7	6.2	
Mudrist odeska	37.1	39.5	22.4	6.0	
Average	37.0	59.2	22.5	6.0	
<b>Rye (winter)</b>					
Knyazhe (control)	43.0	64.8	23.9	7.2	
Kobza	43.7	63.1	25.3	7.9	
Kharkivyanka	41.7	61.9	22.3	7.2	
Average	42.8	63.3	23.8	7.4	
<b>Triticale (winter)</b>					
Markian (control)	45.3	66.2	25.8	9.0	
Molfar	46.7	70.2	28.8	10.2	
Obrij myronivsky	46.1	69.3	27.6	9.9	
Average	46.0	68.6	27.4	9.7	
		Impact intensity	SSD <sub>0.05</sub>	Impact intensity	
				SSD <sub>0.05</sub>	
<b>Factor:</b>					
A (year)	0.82	1.22	0.67	0.30	
B (crop)	0.04	0.94	0.20	0.23	
C (grade)	0.01	0.94	0.02	0.23	
<b>Interaction of factors:</b>					
AB	0.00	2.11	0.01	0.52	
AC	0.00	2.11	0.00	0.52	
BC	0.00	1.63	0.01	0.40	
ABC	0.00	3.65	0.00	0.90	
Other factors	0.13		0.10		

Studies by a number of scientists have confirmed a close correlation between the chlorophyll photosynthetic potential of leaves and grain yield, as follows: 2019 –  $r = 0.99 \pm 0.07$ ; 2020 –  $r = 0.92 \pm 0.22$ ; 2021 –  $r = 0.99 \pm 0.05$ , which is due

to differences in the nature of the relationship of these parameters with weather conditions. The deviations of yields determined by the regression level from the actual value ranged from 0.09 to 1.81 t ha<sup>-1</sup> and averaged about 0.7 t ha<sup>-1</sup> (Pryadkina and Shadchina, 2010; Vozhegova et al, 2019; Voloshchuk and Glyva, 2014; Wu et al, 2012; Wang et al, 2013).

Under the conditions of sufficient moisture in the western forest-steppe zone, it is very important to select for cultivation varieties resistant to ear diseases due to excessive rainfall during the ripening-harvesting period (June–July). According to the average data obtained, the lowest percentage of ear septoria was found in the winter wheat variety – Oberig Myronivsky (2.9%), winter rye – Knyazhe, Kobza (2.4%), winter triticale – Molfar (1.7%) (Table 4).

Table 4. Spread of ear diseases of grain crops depending on varietal characteristics in macrostage 9 BBCH 92 (full maturity) (2019–2021), %.

Variety	Septoria ( <i>Septoria nodorum</i> Berk.)	Fusarium ( <i>Fusarium Link.</i> )		
<b>Soft wheat (winter)</b>				
Vodogray bilotserkivsky (control)	3.3	2.4		
Oberig Myronivsky	2.9	2.1		
Mudrist odeska	3.6	2.7		
Average	3.3	2.4		
<b>Rye (winter)</b>				
Knyazhe (control)	2.2	1.8		
Kobza	2.2	1.8		
Kharkivyanka	2.8	2.0		
Average	2.4	1.9		
<b>Triticale (winter)</b>				
Markian (control)	2.2	2.1		
Molfar	1.7	1.7		
Obrij myronivsky	1.8	1.7		
Average	1.9	1.8		
Factor:	Impact intensity	SSD <sub>0.05</sub>	Impact intensity	SSD <sub>0.05</sub>
A (year)	0.38	0.16	0.52	0.13
B (crop)	0.21	0.12	0.17	0.10
C (grade)	0.12	0.12	0.10	0.10
Interaction of factors:				
AB	0.21	0.27	0.12	0.23
AC	0.02	0.27	0.01	0.23
BC	0.02	0.21	0.02	0.18
ABC	0.02	0.47	0.03	0.40
Other factors	0.01		0.03	

The average percentage of Fusarium spread was 2.4 – soft wheat, 1.9 – rye, 1.8 – triticale. Weather factors had the greatest impact (38 and 52%) on the damage

to winter crop varieties, crop – 21 and 17%, variety – 12 and 10, interaction of all factors – 27 and 18, other factors – 1 and 3%.

Depending on the genetic potential of the variety, its ecological plasticity that allows it to respond positively to the natural and climatic conditions of the study area and the basic cultivation technology used by most small enterprises and farms, we obtained different grain yields over the years (Table 5). It was the highest ( $5.42 \text{ t ha}^{-1}$ ) for soft (winter) wheat in 2019, and the lowest ( $4.98 \text{ t ha}^{-1}$ ) in 2021. It was most favorable for rye (winter) and triticale (winter) in 2020. The range of phenotypic variability for common wheat was  $0.44\text{--}0.71 \text{ t ha}^{-1}$ , for rye –  $0.67\text{--}2.22 \text{ t ha}^{-1}$ , for triticale –  $1.27\text{--}1.37 \text{ t ha}^{-1}$ .

Table 5. Grain yield of winter crop varieties (2019–2021).

Variety	maximum over the test years	Grain yield, $\text{t ha}^{-1}$			average	R (max-min)	% to the maximum
		2018	2020	2021			
<b>Soft wheat (winter)</b>							
Vodogray bilotserkivsky (control)	10.0	5.65	5.48	4.98	5.37	0.67	53.7
Oberig Myronivsky	9.5	5.50	5.56	5.12	5.39	0.44	56.7
Mudrist odeska	11.5	5.10	5.13	4.42	4.88	0.71	42.4
Average	10.3	5.42	5.39	4.84	5.22	0.58	50.7
<b>Rye (winter)</b>							
Knyazhe (control)	7.6	5.15	6.35	5.88	5.79	1.20	76.2
Kobza	9.8	5.25	7.47	6.24	6.32	2.22	64.5
Kharkivyanka	7.5	5.06	5.85	5.73	5.55	0.67	74.0
Average	7.6	5.15	6.55	5.95	5.88	1.36	77.4
<b>Triticale (winter)</b>							
Markian (control)	10.0	5.63	7.00	6.71	6.45	1.37	64.5
Molfar	10.0	5.85	7.12	6.85	6.61	1.27	66.1
Obrij myronivsky	9.7	5.96	7.32	6.95	6.74	1.36	69.5
Average	9.9	5.81	7.15	6.84	6.60	1.34	66.7
Factor:	Impact intensity			$\text{SSD}_{0.05}$			
A (year)	0.43			0.17			
B (crop)	0.22			0.15			
C (grade)	0.16			0.15			
<b>Interaction of factors:</b>							
AB	0.07			0.29			
AC	0.04			0.29			
BC	0.04			0.23			
ABC	0.03			0.49			
Other factors	0.01						

Comparing the yield actually obtained by the studied winter crop varieties with the maximum productivity in the test years, we determined the level of

implementation in the conditions of the studied soil-climatic zone. Thus, for soft wheat, it was 42.4% for the Mudrist odeska variety – 56.7 t ha<sup>-1</sup> Oberig Myronivsky; for rye – 64.5 (Kobza) – 76.2 t ha<sup>-1</sup> (Knyazhe), and for triticale – 64.5 (Markiyan) – 69.5 t ha<sup>-1</sup> (Obrij myronivsky), which indicates the still completely unused genetic potential.

The influence of weather factors on the grain productivity of winter crop varieties was 44.0%, crops – 22.0, varieties – 16, their interaction – 18.0, the rest – 1.0%.

The massive spread of the phenomenon of enzymomycotic depletion of grain (EMDG) has attracted renewed attention in recent years, especially in the western forest-steppe and Polissya. The disease is more prevalent in those years when there is excessive rainfall, frequent fog formation, and air temperatures above 30 °C during the waxy and full ripening of the grain. Drop moisture penetrates into the middle of the grain, causes the hydrolysis of carbohydrates, increases the osmotic pressure in the grain cells and enhances water endosmosis. As a result of the increasing hydrostatic pressure, microscopic ruptures form in the cell walls and grain shells. These gaps produce a sugary liquid on the surface of the grain, which is washed off by raindrops on the spikelet scales and the upper parts of the stem. Fungi of the genera *Alternaria* and *Cladosporium* colonize these secretions, enhancing the development of black germ and olive mold. Enzymomycotic depletion of the grain causes a reduction in wheat yield by 20–30% or more (Vorobyova, 2016).

According to the research of Pogorila et al. (2019), seeds affected by *Alternaria* are physiologically underdeveloped. They have low germination vigor and similarity. Plants grown from such seeds lag behind in growth and development, resulting in lower yields. Grain flour with a “black germ” has a dark color and poor baking properties.

An important task for the producers of grain products is to prevent or minimize shortfalls and losses in the crop and its quality.

This problem is especially aggravated when the harvesting of winter grain crops is delayed up to 12 days after the onset of full grain maturity due to the lack of harvesting equipment.

In our experiments, the weight of 1000 grains of winter wheat at the stage of full maturity varied from 42.7 g for the variety of the ecological steppe type Mudrist odeska to 44.5 g for the forest-steppe variety Oberig Myronivsky (Table 6). On the 4<sup>th</sup> day after the onset of full maturity in these varieties, this indicator decreased by 3.3 and 2.5% for these varieties, – by 5.0 and 3.7% g on the eighth day, and by 6.8 and 5.3% on the twelfth day. Compared to wheat, winter rye varieties are characterized by higher average weight loss per 1000 grains – 5.0–10.3%.

Table 6. Weight loss of 1000 grains of winter crop varieties after full maturity (2019–2021).

Variety	Full maturity		Grain weight loss per day, g %		
	g	± to control	4	8	12
<b>Soft wheat (winter)</b>					
Vodogray bilotserkivsky (control)	43.5	-	1.0/3.0	1.9/4.4	2.6/6.0
Oberig Myronivsky	44.5	1.0	1.1/2.5	1.6/3.7	2.3/5.3
Mudrist odeska	42.7	-0.8	1.6/3.3	2.2/5.0	2.9/6.8
Average	43.6		1.2/2.9	1.9/4.4	2.6/6.0
<b>Rye (winter)</b>					
Knyazhe (control)	35.4	-	1.7/4.8	2.6/7.4	3.6/10.3
Kobza	36.4	1.0	1.7/4.6	2.3/6.5	3.3/9.3
Kharkivyanka	34.0	-1.4	1.9/5.6	2.8/8.3	3.8/11.2
Average	35.3		1.8/5.0	2.6/7.4	3.6/10.3
<b>Triticale (winter)</b>					
Markian (control)	44.0	-	1.4/3.3	2.2/5.0	3.0/6.9
Molfar	45.6	1.6	1.4/3.1	2.1/4.6	2.3/6.4
Obrij myronivsky	45.7	1.7	1.4/3.1	2.0/4.4	2.9/6.4
Average	45.1		1.4/3.2	2.1/4.7	2.7/6.6
Factor:	Impact intensity		$SSD_{0.05}$		
A (year)	0.45		0.24		
B (crop)	0.18		0.19		
C (grade)	0.15		0.19		
Interaction of factors:					
AB	0.06		0.42		
AC	0.04		0.42		
BC	0.07		0.32		
ABC	0.03		0.72		
Other factors	0.02				

The data of the analysis confirm that the years of research had the greatest impact on the phenomenon of enzymomycotic depletion of grain - 45%; the plant - 18% and the variety - 15%, to a lesser extent.

Climate change puts additional pressure on agricultural producers, especially the most vulnerable smallholder farms not only in Ukraine, but worldwide (Mavromatis, 2015; Izumi et al., 2018; Hochman et al., 2017; Gupta et al., 2017; Donatti et al., 2019).

To solve the problem of food security in any country, much attention is paid to the use of new varieties that provide higher gross yields by 20–50%. Research by Chinese scientists confirms that the yield potential depends on a variety adapted to an environment with unlimited nutrients, water, effective control of pests and diseases, and exploits the yield potential of rice by more than 80%. Breeders consider it a priority to reduce the yield gap between corn and wheat through new varieties (Fan et al., 2012).

According to Ceglara et al. (2019), the gaps in the yield of grain crops range from 10 to 70% in the world, being small in many countries of North-Western Europe and large in Eastern and South-Western Europe. They are lower for rainfed and irrigated corn than for wheat and barley. The potential for increasing grain production is in Eastern Europe, half of which is in Ukraine, Romania and Poland.

### Conclusion

With the basic growing technology and favorable weather conditions, winter crop varieties had optimal development at the end of the fall vegetation, and accumulated a sufficient amount of sugars in the tillering nodes (26.9–27.1%), which contributed to a high percentage of overwintering (96.6 – soft wheat [winter], 94.9 – sowing rye [winter], 94.2 – triticale [winter]). The largest total leaf surface area in the third macrostage BBCH 37–39 was formed by varieties: soft winter wheat – Oberig myronivsky (61.5 thousand  $m^2 ha^{-1}$ ), rye – Knyazhe (64.8 thousand  $m^2 ha^{-1}$ ), triticale – Molfar (70.2 thousand  $m^2 ha^{-1}$ ). The average degree of spread of ear diseases was as follows: soft winter wheat – 3.3% (septoria), 2.4% (fusarium); rye – 2.4 and 1.9%, respectively, winter triticale – 1.9 and 1.8%, respectively. The grain yield of soft winter wheat varieties ranged from 4.88 (*Mudrist odeska*) to 5.39  $t ha^{-1}$  (*Oberig myronivsky*); rye – 5.55 (*Kharkivyanka*) – 6.32  $t ha^{-1}$  (*Kobza*); triticale – 6.45 (*Markiyan*) – 6.74  $t ha^{-1}$  (*Obrij myronivsky*). Grain productivity of soft winter wheat varieties varied from 4.88  $t ha^{-1}$  (*Mudrist odeska*) to 5.39  $t ha^{-1}$  (*Oberig myronivsky*); rye – from 5.55  $t ha^{-1}$  (*Kharkovyanka*) – to 6.32  $t ha^{-1}$  (*Kobza*); triticale – from 6.45  $t ha^{-1}$  (*Markiyan*) – to 6.74  $t ha^{-1}$  (*Obrij myronivsky*). When overstaying grain “on the root” for 12 days, the average weight loss of 1000 grains for varieties of soft wheat (winter) was – 6.0%, for triticale (winter) – 6.6%, and for rye (winter) – 10.5%.

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R e z i m e

Cilj rada je bio da se odrede parametri prilagodljivosti sorti ozimih kultura za gajenje u zapadnoj šumno-stepskoj zoni Ukrajine (2019–2021). Predmet istraživanja bile su sorte pšenice (*Triticum aestivum L.*): Vodogray bilotserkivsky, Oberig Myronivsky, Mudrist odeska; raži (*Secale sereale L.*): Knyazhe, Kobza, Kharkivyanka; i tritikalea (*Triticosecale Witt.*): Markian, Molfar, Obrij myronivsky. Ispitivanja su obavljena u uslovima poljskog ogleda. Površina ogledne parcele iznosila je 55 m<sup>2</sup>, a ogled je bio postavljen u tri ponavljanja. Rezultati su pokazali da je u skladu sa osnovnim tehnologijama gajenja i vremenskim uslovima tokom vegetacionog perioda, produktivnost sorti meke pšenice varirala od 4,88 (Mudrist odeska) do 5,39 t ha<sup>-1</sup> (Oberig Myronivsky); raži – od 5,55 (Kharkivyanka) do 6,32 t ha<sup>-1</sup> (Kobza); i tritikalea – od 6,45 (Markian) do 6,74 t ha<sup>-1</sup> (Obrij myronivsky). Dobijeni rezultati dovode do zaključka da ekološki plastične sorte ozimih useva u uslovima proučavane zemljишno-klimatske zone akumuliraju dovoljnu količinu šećera u čvorovima bokorenja - 25–30%, osiguravaju prezimljavanje biljaka - 94–96%, otporne su na glavne bolesti, što doprinosi proizvodnji visokokvalitetnog semena za snabdevanje obradivih površina u regionu i efikasnost semenske proizvodnje.

**Ključne reči:** bolest, ozima meka pšenica, ozima raž, ozimi tritikale, masa 1000 zrna, prinos.

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## THE RELATIONSHIP BETWEEN THE END OF THE VEGETATION PERIOD AND FRUIT PROPERTIES IN THE WALNUT POPULATION IN EASTERN SERBIA

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**Abstract:** The main objective of the study was to examine the walnut population from the region of Eastern Serbia for determining the relationship between the end of the vegetation period and fruit properties (fruit weight, kernel percentage, oil and crude protein contents). The walnut trees showed highly significant differences in the tested parameters. The walnut population was dominated by trees with a medium end of the vegetation period, moderate resistance to low temperatures (39.5%) and very small fruits (23.5%). The fruits of these trees had a high kernel percentage (15.8%) and moderate oil and crude protein contents (25.8% and 38.3%, respectively). Contrary, the walnut population comprised the fewest trees of late ending of the vegetation period, with extremely large fruits (0.1%), exceptional kernel percentage (0.2%) and pronouncedly high oil and crude protein contents (0.07% and 0.5%, respectively). The studied walnut trees show great variability in the date of the end of vegetation and fruit properties, which indicates a high potential of the population in the selection of new genotypes, and the necessity for their conservation for further breeding, propagation and commercial cultivation.

**Key words:** *Juglans regia* L., end of the vegetation period, fruit characteristics.

### Introduction

In Serbia, the natural walnut populations are highly diverse since walnut has been propagated by seeds for years. Generative propagation accounts for the populations with pronounced biotype polymorphism, which makes the walnut a monoecious, anemophilous and dichogamous fruit species. The existence of natural walnut populations is very important, because it enables the collection, selection and hybridisation of genotypes, which therefore ensures a high quality selection aimed at breeding new cultivars.

Within phenological studies, the end of the growing season is the one of the most significant phases in the life of the walnut and is closely associated with the

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period of dormancy. A favourable time of the end of the growing season ensures good plant performance during dormancy and its resistance to low winter temperatures (Miletić, 2009). Korać and Cerović (1980) reported that in the walnut population there is a positive correlation between the time of leaf bud burst and the time of leaf fall, as well as a correlation between the end of the growing season and resistance to low winter temperatures. Therefore, the end of vegetation before the first autumn frost is an important trait for all plant populations, including walnut (Korać et al., 1990).

Additionally, high variability in fruit characteristics has been recorded in the walnut populations. Zeneli et al. (2005) and Sharma et al. (2010) noted a high range of variability observed for various fruit characters in native walnut populations. Also, Cerović et al. (2017) found that walnut populations in various parts of the world show not only great variability in the duration of the vegetative period and the time of leaf fall, but also in fruit weight, kernel percentage, etc.

Given the above, the objective of this investigation was to determine the relationship between the end of the vegetation period and major fruit characteristics (fruit weight, kernel percentage, oil and crude protein contents) in walnut seedlings from the natural population.

## Material and Methods

The study was carried out on five localities (Zaječar, Knjaževac, Negotin, Mali Izvor and Sokobanja) in Eastern Serbia, during 2008–2010. Over 1,464 walnut trees of generative origin were randomly collected. All the trees studied were of seedling origin.

The paper shows the end of the vegetation period of walnut and the main fruit properties. The description of the fruit characteristics is given based on the descriptor for the walnut from the International Union for the Protection of New Varieties of Plants (UPOV, 1999).

Fruit weight was classified in five categories: very small ( $>8.0$  g), small (8.1–10.0 g), mid-large (10.1–12.0 g), large (12.1–14.0 g) and very large ( $<14.1$  g), while kernel percentage was presented in seven categories: extremely low ( $>30\%$ ), very low (30.1–35.0%), low (35.1–40.0%), medium (40.1–45.0%), high (45.1–50.0%), very high (50.1–55.0%) and extremely high ( $<55.1\%$ ). Fruit weight was measured by using a 'Mettler' technical scale. Kernel percentage was calculated based on fruit and kernel weight.

The kernel oil content was determined by the NMR (nuclear magnetic resonance) method (AOAC, 1995) and presented in four categories: low ( $>50\%$ ), medium (50.1–60.0%), high (60.1–70.0%) and very high ( $<70.1\%$ ). The crude protein content was identified by the micro-Kjeldahl method (Jung et al., 2003), and the samples were classified in three categories: kernel with low (up to 15.0%), medium (15.1–20.0%) and high crude protein content ( $>20.1\%$ ).

The time of the end of the growing season is presented in three categories: early (1), medium (2) and late (3). The resistance of walnut trees to low winter temperatures was determined based on the percentage of frozen buds during the winter. The assessment of the damage caused by the low winter temperatures included examining of the top and lateral buds, plant monitoring before the growing period onset, and examining the results during the growing period onset (the number of inactivated buds). According to the results, walnut trees are described as those with high resistance (1), moderate resistance (2), and low resistance (3), respectively, to low winter temperatures.

The paper presents the average three-year results, including the share (%) of each trait in the total number of trees. The data obtained were statistically processed by analysis of variance and the Duncan's multiple range test.

## Results and Discussion

Non-uniform flowering time and inflorescence development encourage cross-pollination in the walnut, which induces the development of an abundant and heterogeneous population exhibiting different pomological properties (Miletić, 2004). Regardless of the pronounced heterogeneity within the walnut population, there is a considerable relationship among particular phenophases, given that the span of the growing season may be the limiting factor to the overall performance of the walnut tree (Miletić, 2009; Miletić et al., 2010). The studied population was dominated by trees with a medium time of leaf fall (52.39%), then with an early time of leaf fall (34.97%), whereas a late time of leaf fall was the least prevalent (12.64%). These data are consistent with the results of Korać et al. (1990), who found that walnut populations are dominated by trees with a medium end of the growing season (55%), followed by those with an early period (28%), while trees with the late end of vegetation are the least prevalent (17%). Moreover, Cerović et al. (2017) indicated that the walnut population in the Western Balkans has the most genotypes of the early time of leaf fall (about 51%), and the fewest genotypes with a late time of leaf fall (about 17%).

Table 1. The relationship between the end of the growing season and the resistance to low winter temperatures.

End of vegetation	Trees in the population (%)			
	High resistance	Moderate resistance	Low resistance	Total (%)
Early	1.09	25.68	8.2	34.97
Medium	0.89	39.50	12.0	52.39
Late	0.14	5.30	7.2	12.64
Total (%)	2.12	70.48	27.4	100.0

With regard to low winter temperatures, the fewest were the trees exhibiting high resistance to low winter temperatures (2.12%), followed by low-resistant trees (27.4%), whereas there were mostly trees of medium resistance (70.48%). Generally, in the walnut population, the most prevalent genotypes were those with the medium end time of the vegetation and moderate resistance to low winter temperatures, while the smallest number of trees were those with the late end of the vegetation and high resistance. Agro-environmental conditions have a major impact on walnut performance in natural populations (Korać, 1998). A new set of genes is thus created and populations change to adapt to the existing environmental conditions (Borojević, 1986).

Fruit quality in walnut populations is quite non-uniform, which is the result of un-cultivated production. In the present study, the fruits were mainly very small and small, whereas those with large and very large fruits were in the minority (Table 2).

Table 2. The relationship between the end of the vegetation period and the fruit weight of the walnut (number of trees in the population in %).

End of vegetation	Fruit weight (g)					Total (%)
	>8.0	8.1–10.0	10.1–12.0	12.1–14.0	<14.1	
Early	16.0	12.4	5.6	1.8	1.6	37.4
Medium	23.5	16.0	7.5	2.0	1.0	50.0
Late	7.2	3.9	0.9	0.5	0.1	12.6
Total (%)	46.7	32.3	14.0	4.3	2.7	100.0

Trees of the medium ending of the vegetation had very small (23.5%) and small fruits (16.0%), while the percentage of large and very large fruits was significantly lower (2.0% and 1.0%, respectively). In trees that early end the vegetation period, the percentage of small and very small fruits (16.0% and 12.4%, respectively), as well as of large and very large fruits (1.8% and 1.6%, respectively), was higher than in the mid-season trees. The lowest percentage of very small and small fruits (7.2% and 3.9%, respectively), and of large and very large fruits (0.5% and 0.1%, respectively) was recorded in trees whose vegetation period end quite late. A considerable percentage of trees with small and very small fruits is indicative of population specificity and its high variability, which is comparable to the research by Korać et al. (1990), who reported that the walnut population grown on Fruška Gora is dominated by trees with small fruits (2.2%), while those with very large fruits are the fewest (7.2%). Also, Cerović et al. (2017) observed that most genotypes in the walnut population have very small (28.6%) and small (32.2%) fruit sizes. Namely, Zeneli et al. (2005) indicated that, in native trees which were of seedling origin, significant genetic variation was found in pomological characteristics, with fruit weights ranging from 3.8 to 21.1 g.

In terms of kernel percentage, the data in Table 3 reveal that fruits with medium, high and low kernel contents (29.0%, 28.7% and 16.0%, respectively) were predominant in the population studied, whereas the share of fruits with extremely low kernel content was the lowest (2.2%). Fruits with medium kernel percentage were mostly found in trees with the early ending of vegetation, while trees with mid-season ending were dominated by fruits with medium, low and high kernel percentages. Kernel percentage in walnut trees with the late ending of the growing season varied from 0.2 to 3.9%.

Table 3. The relationship between the end of the vegetation period and kernel percentage in walnut fruits (number of trees in the population in %).

End of vegetation	Kernel percentage (%)							Total (%)
	>30.0	30.1–35.0	35.1–40.0	40.1–45.0	45.1–50.0	50.1–55.0	<55.1	
Early	0.8	1.7	5.1	11.1	11.3	6.1	1.3	37.4
Medium	0.9	3.5	7.0	14.6	15.8	8.1	2.0	51.9
Late	0.5	0.2	3.9	3.3	1.6	1.0	0.2	10.7
Total (%)	2.2	5.4	16.0	29.0	28.7	15.2	3.5	100.0

Cerović et al. (2017) reported that only one third of genotypes have a kernel percentage of 40% and above, while 9.3% have high and very high kernel percentages. According to Paunović and Miletić (2013), walnut populations consist of genotypes with kernel percentages ranging from 20.2% to 65.6%. Many studies have found similar variability in fruit weight and kernel weight, as well as kernel percentage in fruits from the natural walnut populations (Sharma and Sharma, 2001; Dragănescu et al., 2001; Botu et al., 2001).

With respect to oil content, the obtained results show that there is a significant relationship between the end of the vegetation period and the oil content in the kernel (Table 4).

Table 4. The relationship between the end of the vegetation period and the oil content in the kernel (number of trees in the population in %).

End of vegetation	Oil content (%)				Total (%)
	up to 50.0	50.1–60.0	60.1–70.0	<70.1	
Early	1.8	17.7	15.4	0.5	35.4
Medium	2.4	25.8	22.9	0.6	51.7
Late	1.0	7.03	4.8	0.07	12.9
Total (%)	5.2	50.53	43.1	1.17	100.0

The results show that most of the fruits were evidenced with medium (50.53%) and high oil content in the kernel (43.1%), while fruits with low (5.2%) and very high oil content (1.17%) were fewer. The total oil content in the fruits

was the highest in trees of the medium ending of vegetation (51.7%), and the lowest in those of the late ending of vegetation (12.9%).

Regarding crude protein content, fruits with medium protein content predominated in all categories studied (Table 5). The highest total protein content in the kernel was found in trees of the medium ending of the vegetation season (53.6%), and the lowest in fruits with the late ending of vegetation (12.2%), which is consistent with the results of Ozkan and Koyuncu (2005), Sharma et al. (2014) and Jaćimović et al. (2020), who recorded that in the walnut population the average crude protein content in the kernels ranges from 13.6% to 19.2%, while the oil content varies from 62.0% to 70.9%. According to Miletić (2004), the oil content in the walnut kernel is higher in dry years and lower in years with heavier rainfall, while the opposite regularity is observed for the crude protein content in the kernel.

Table 5. The relationship between the end of the vegetation period and the crude protein content in the kernel (number of trees in the population in %).

End of vegetation	Protein content (%)			Total (%)
	up to 15.0	15.1–20.0	<20.1	
Early	8.8	23.4	2.0	34.2
Medium	12.6	38.3	2.7	53.6
Late	2.6	9.1	0.5	12.2
Total (%)	24.0	70.8	5.2	100.0

## Conclusion

The results of the present research reveal that there is no strict relationship between the end of the vegetation period and fruit characteristics. The walnut population was dominated by trees with the medium ending of vegetation and moderate resistance to low temperatures, which can be considered an important characteristic of the population. In this way, good conditions for winter dormancy and higher resistance to low winter temperatures are provided, preventing damage due to late autumn frosts.

In all studied categories, the fruits were predominantly very small and small, while large and very large fruits were found on a small number of trees. Fruits with high and medium kernel percentages accounted for 57.7% of the total number of trees, which is a very significant property and an important parameter of the quality of the walnut population, especially from the standpoint of economics.

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ODNOS ZAVRŠETKA VEGETACIJE I OSOBINA PLODA U  
POPULACIJI ORAHA U ISTOČNOJ SRBIJI

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R e z i m e

Osnovni cilj istraživanja bio je proučavanje populacije oraha sa područja istočne Srbije radi utvrđivanja odnosa između završetka vegetacionog perioda i osobina ploda (masa ploda, sadržaj jezgre, sadržaj ulja i sirovih proteina). Stabla oraha su pokazala značajne razlike u ispitivanim parametrima. U populaciji oraha dominirala su stabla srednjeg vremena završetka vegetacije, umerene otpornosti prema niskim zimskim temperaturama (39,5%) i veoma sitnih plodova (23,5%). Plodovi ovih stabala imali su veliki randman jezgre (15,8%), i srednji sadržaj ulja (25,8%) i sirovih proteina (38,3%). Nasuprot tome, u populaciji je bilo najmanje stabala oraha pozognog vremena završetka vegetacije, sa ekstremno krupnim plodovima (0,1%), visokim randmanom jezgre (0,2%) i izrazito visokim sadržajem ulja (0,07%) i sirovih proteina (0,5%). Proučavana stabla oraha pokazuju veliku varijabilnost u pogledu vremena završetka vegetacije i osobina ploda, što ukazuje na visok potencijal populacije u selekciji novih genotipova, kao i na neophodnost njihovog očuvanja u cilju daljeg oplemenjivanja, razmnožavanja i komercijalnog gajenja.

**Ključne reči:** *Juglans regia* L., završetak vegetacije, karakteristike ploda.

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## THE IMPROVEMENT OF THE BODY AND UDDER HYGIENE INFLUENCE ON THE MILK SAFETY AND COMPOSITION ON SMALL DAIRY F FARMS IN SERBIA

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**Abstract:** The hygienic condition of dairy cows and their udders in 128 small household farms in different regions of Serbia, producing mostly milk, having 5–15 cows, a milk cooler and at least one milking machine, was evaluated by the following indicators: 1. general assessment of body cleanliness, 2. visual inspection of teats and udder base, and 3. maintenance of udder cleanliness, rated on a scale of 0 and 1 or 1 to 5. The samples were taken at each visit to the farm after cooling and tested for milk protein and fat content, somatic cell count (SCC) and the total number of microorganisms (TNM). The milk fat content was determined by the Gerber method, the protein content by the Kjeldahl method, the TNM according to the ISO 4833-1:2013 method and the SCC using Fossomatic TM. The IBM SPSS program was used for statistical data processing. Capacity, housing system, breed, milking system, number of cows, and capacity occupancy mostly had a very significant or significant impact on hygiene parameters, protein and fat content, SCC and TNM in milk. The hygiene parameters showed a continuous trend of improvement in relation to the visits. TNM mean scores increased after each visit, with significant differences between visits ( $F=9.63$ ,  $P<0.0001$ ). SCC scores varied very significantly between visits ( $F=5.17$ ,  $P<0.0001$ ). The number of visits tended to show a significant influence on the milk fat rate ( $F=2.221$ ;  $P<0.1$ ), but no influence on the milk protein rate ( $P=0.480$ ;  $F=0.901$ ).

**Key words:** improvement, body, udder, hygiene, milk, safety, composition, small dairy farms.

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## Introduction

Milk production is an important financial resource for the survival and life of small farmers and their families. Today, it is increasingly important for them to produce milk and dairy products that meet food safety standards and to take advantage of the growing demand for their products.

Therefore, research often examines the relationship between the hygiene parameters of the farm, the cows and the milkers with the hygiene and milk composition. Success in milk production and processing practically depends on the adequate implementation of hygiene measures before and during milking and the procedures with the milk immediately after milking (Tamime, 2009; Oumer et al., 2017; Berge and Baars, 2020). These areas are closely related and are particularly evident in cows on small and medium-sized family farms.

The literature indicates that a clean cow body is an important factor in obtaining and maintaining milk quality (Schreiner and Ruegg, 2002; Sant'anna and da Costa, 2011; Robles et al., 2020). When cows lie on dirty bedding, feces, urine, soiled mats, and feed stick to the cow's body. During milking, this muck from the cow's body may easily get into the milking machines and milk. Regular removal of bedding and daily care of the cow's body may reduce the contamination of the cow's body and thus the milk contamination, especially on the parts of the body that are important for milking hygiene: hindquarters, udder, teats, abdomen, groin and extremities. A new mat should be added daily to keep the lying area clean and dry (Hristov, 2002; Relić and Hristov, 2016).

Maintaining animal health is extremely important for hygienic milk production (Schukken et al., 2003; Malik et al., 2018), whereby special attention should be paid to the occurrence of udder infections that lead to contamination of milk through the teats, and gastrointestinal infections increase the contamination of teat surfaces.

## Material and Methods

During the study, the hygienic condition of dairy cows and their udders on farms was evaluated using a defined questionnaire, and sampled milk was tested for protein and milk fat content, SCC and the total number of microorganisms (TNM). On-farm examinations were performed three to six times, depending on whether on-farm hygiene measures needed to be corrected. The assessment and analysis of the situation were carried out on 128 small farms with free or tied rearing in different regions of Serbia. The selection of farms was made by the advisers of the Agricultural Advisory and Expert Services (AAES) of Požarevac, Jagodina, Kragujevac, Kruševac, Negotin, Niš, Prokuplje, Leskovac, Vranje and Pirot. The prerequisite was that the farmers are predominantly engaged in milk production and possess 5–15 cows, a milk cooler tank and at least one mechanical

milking machine. The structure of the questionnaire was defined based on the methods of Kurwijila (2006), Cook and Reinemann (2007), Atasever et al. (2012), De Vries et al. (2012), and Relić and Hristov (2016), with the necessary modifications for a more detailed overview for the assessment of hygiene of cows, milking units as well as the procedures during milking. The questions corresponded to indicators and were rated on a scale of 0 and 1 or 1 to 5 (1 – poor, 2 – sufficient, 3 – good, 4 – very good and 5 – excellent). Based on the analysis of the obtained results, recommendations for correcting the hygienic procedures in milking were defined and agreed with the farmers. Questions 1–3 of the questionnaire were related to: 1. general assessment of body cleanliness, 2. visual inspection of teats and udder base for soiling and 3. maintaining of teat cleanliness visually or by using wet wipes or cotton wool.

For the application of the proposed corrective hygiene measures to improve hygienic conditions before, during and after milking of cows, farmers were trained by advisors with additional printed instructional materials.

The samples were collected after cooling of the total amount of milk and delivered to the selected accredited laboratory for analysis. These samples were taken at each farm visit, which makes a total of six samples per farm during the study period. The milk fat content was determined by the Gerber method (Anon., 2018), the protein content by the Kjeldahl method (Anon., 2014), the TNM value according to the ISO 4833-1:2013 method (Anon., 2013) and the SCC value by the fluoro-opto-electronic method on the Fossomatic TM apparatus and by the method of cytological staining of milk cells and counting under a microscope.

The IBM SPSS program was used for statistical data processing with a descriptive presentation of farm characteristics, scores for all questions of the questionnaire and value and score for milk composition, considering average scores, differences and proportions of scores, using ANOVA analysis and  $\chi^2$  test. To examine the relationship between the farm characteristics and the milk quality scores and the questions from the questionnaire, the size of the effect of the farm factors on the mentioned scores was determined using a partial  $\eta^2$  test.

## Results and Discussion

Analyzing the general score of body cleanliness at the herd level in Table 1, the most common score determined was 4 with 45.7%, followed by score 3 with 31.9%.

When evaluating body hygiene at the herd level, the score assigned to the largest number of examined animals was taken into account, as well as the average score. The most frequently obtained score of teat and udder base cleanliness by visual inspection was 5 (65.2%), while the scores for classes 3 and 4 were very similar (13.8% and 15.9%, respectively, see Table 2).

Table 1. General evaluation of the cows' body cleanliness.

	Parameter	Frequency	%	Cumulative %
Score	2	56	8.7	8.7
	3	206	31.9	40.6
	4	295	45.7	86.2
	5	89	13.8	100
	$\Sigma$	646	100	100

Table 2. The inspection of teats and udder bases for soiling.

	Parameter	Frequency	%	Cumulative %
Score	1	14	2.2	2.2
	2	19	2.9	5.1
	3	89	13.8	18.9
	4	103	15.9	34.8
	5	421	65.2	100
	$\Sigma$	646	100	100

Regarding the hygiene of the teats (Table 3), which was determined visually and by using wet wipes or cotton wool to wipe the top of the teat, as well as by assigning the appropriate scores taking into account the number and percentage of teats whose tips were dirty, the most common score was 3 (good) amounting to 41.6%, followed by the percentage representation of score 4 (29.1%) and score 5 (24.5%).

Table 3. Maintenance of teat cleanliness.

	Parameter	Frequency	%	Cumulative %
Score	1	11	1.7	1.7
	2	20	3.1	4.8
	3	269	41.6	46.4
	4	188	29.1	75.5
	5	158	24.5	100
	$\Sigma$	646	100	100

Table 4 shows the univariate analysis of variance for the results related to the body hygiene parameters at the herd level (as a criterion, the score assigned to the largest number of examined animals and the average score) as hygiene measures.

The capacity, rearing system and capacity occupancy had a significant impact on cow body hygiene and daily milk production. Other parameters did not have a statistically significant influence on cow body hygiene. The partial  $\eta^2$  test

revealed that the greatest impacts on daily milk production, capacity occupancy and capacity were 19.2%, 13.2% and 10.4%, respectively.

Table 4. Univariate analysis of variance for the assessment of the parameters of body hygiene of cows.

Dependent variable	Tests of effects between factors and parameters					
	Question 1					
Source	Sum of squares type III	df	Average square	F	Significance	$\eta^2$
Corrected model	225.435a	200	1.127	2.362	0.000	0.515
Section	24.689	1	24.689	51.730	0.000	0.104
Capacity	24.755	14	1.768	3.705	0.000	0.104
Rearing system	7.526	1	7.526	15.768	0.000	0.034
Breed	0.248	1	0.248	0.519	0.471	0,001
Milking system	0.834	1	0.834	1.747	0.187	0.004
No. of cows in a facility	7.300	10	0.730	1.530	0.126	0.033
Capacity utilization	32.207	32	1.006	2.109	0.001	0.132
No. of cows milked	11.308	16	0.707	1.481	0.102	0.051
Daily milk production	50.385	75	0,672	1.408	0.020	0.192
Daily milk production per cow	15.614	25	0.625	1.309	0.147	0.068
Error	212.387	445	0.477			
Total	9023.000	646				
Total corrected	437.822	645				

a.  $R^2 = 0.515$  (Adjusted  $R^2 = 0.297$ ).

Table 5 shows the univariate analysis of variance for the results related to the parameter considering whether the teats and the base of the udder are visually inspected.

A highly significant influence for the visual inspection of teats and udder base and their soiling was found for capacity, milking system, capacity occupancy, daily milk production, daily milk production per cow, and number of dairy cows. Other parameters did not show significance for the visual examination of the teats and the base of the udder and their soiling. Taking into account the partial  $\eta^2$  value, the greatest impact on the visual inspection of the teat and the base of the udder and their soiling was found for daily milk production (27.0%) and capacity occupancy (15.0%).

Table 6 shows the univariate analysis of variance for the results related to the parameter of maintaining teat cleanliness (determining visually or using wet wipes or cotton wool, which are used to wipe the top of the teat and evaluate accordingly; for the teat cleanliness rating, it was important to determine the number and percentage of teats whose tips were soiled), as hygiene measures in the conducted tests.

Table 5. Univariate analysis of variance for the assessment of teat and udder base cleanliness.

Dependent variable	Tests of effects between factors and parameters					
	Question 2					
Source	Sum of squares type III	df	Average square	F	Significance	$\eta^2$
Corrected model	384.176 <sup>a</sup>	200	1.921	3.724	0.000	0.626
Section	62.548	1	62.548	121.269	0.000	0.214
Capacity	21.432	14	1.531	2.968	0.000	0.085
Rearing system	1.921	1	1.921	3.724	0.054	0.008
Breed	1.465	1	1.465	2.840	0.093	0.006
Milking system	5.938	1	5.938	11.514	0.001	0.025
No. of cows in the facility	8.359	10	0.836	1.621	0.098	0.035
Capacity utilization	40.467	32	1.265	2.452	0.000	0.150
No. of cows milked	17.460	16	1.091	2.116	0.007	0.071
Daily milk production	84.896	75	1.132	2.195	0.000	0.270
Daily production per cow	25.182	25	1.007	1.953	0.004	0.099
Error	229.521	445	0.516			
Total	13064.000	646				
Total corrected	613.697	645				

a.  $R^2 = 0.626$  (Adjusted  $R^2 = 0.458$ ).

Table 6. Univariate analysis of variance for the evaluation of the teat cleanliness.

Dependent variable	Tests of effects between factors and parameters					
	Question 3					
Source	Sum of squares type III	df	Average square	F	Significance	$\eta^2$
Corrected model	334.680 <sup>a</sup>	200	1.673	3.402	0.000	0.605
Section	34.006	1	34.006	69.127	0.000	0.134
Capacity	23.282	14	1.663	3.381	0.000	0.096
Rearing system	0.459	1	0.459	0.934	0.334	0.002
Breed	1.477	1	1.477	3.003	0.084	0.007
Milking system	2.018	1	2.018	4.103	0.043	0.009
No. of cows in the facility	6.219	10	0.622	1.264	0.248	0.028
Capacity utilization	43.730	32	1.367	2.778	0.000	0.167
No. of cows milked	21.230	16	1.327	2.697	0.000	0.088
Daily milk production	99.206	75	1.323	2.689	0.000	0.312
Daily milk production per cow	25.534	25	1.021	2.076	0.002	0.104
Error	218.911	445	0.492			
Total	9470.000	646				
Total corrected	553.591	645				

a.  $R^2 = 0.605$  ( $R^2 = 0.427$ ).

In the category of parameters related to the hygiene of the cow's body, the general evaluation of the cleanliness of the body, the visual inspection of the teats and the base of the udders for soiling and the maintenance of the cleanliness of the teats were considered. The results of the test showed that during the implementation of the hygiene measures there was an increase in the scores for the general evaluation of the cleanliness of the cows' bodies (Figure 1) from the first to the sixth farm visit ( $F=21.47$ ,  $P<0.0001$ ). The increase resulted from a decrease in scores in the range 1–3 (presence of dried and fresh faeces) and an increase in the frequency of scores 4 and 5, indicating slight soiling or a clean body ( $\chi^2 = 100.38$ ,  $P<0.0001$ ). In addition, the results showed that the value of scores for the visual inspection of the teats and the base of the udder for soiling during the implementation of hygiene measures (Figure 2) increased from the first to the sixth visit to the farm ( $F=8.83$ ,  $P<0.0001$ ). This increase resulted from a decrease in the frequency of scores indicating that the teats and the base of the udder were not visually inspected for soiling and an increase in the frequency of scores indicating that this inspection was performed daily ( $\chi^2 = 66.83$ ,  $P<0.0001$ ). When examining the parameter of maintaining the cleanliness of the teats during the implementation of hygiene measures, an increase in the frequency value and rating for maintaining the cleanliness of the teats (Figure 3) was observed from the first to the sixth visit to the farm ( $F=13.12$ ,  $P<0.0001$ ). It was observed that the increase was due to the decrease in the frequency of scores 1 (the presence of a greater amount of older, neglected, dried dirt), 2 (a greater amount of dirt on the teats) and 3 (a smaller amount of dirt on the teats), and an increase in the frequency of score 4 (traces of disinfectant, without traces of impurities) and score 5, indicating the absence of traces of disinfectant colour or impurities ( $\chi^2 = 76.30$ ,  $P<0.0001$ ).

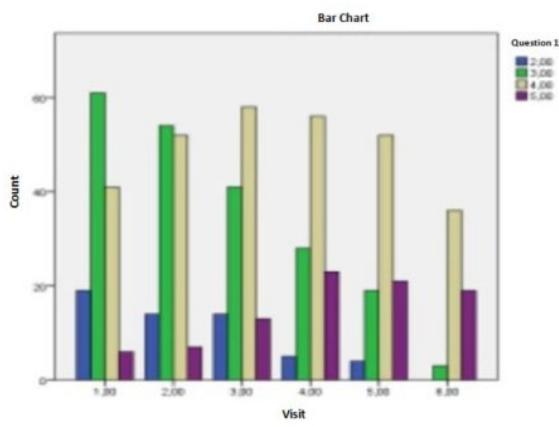


Figure 1. General assessment of body cleanliness during visits.

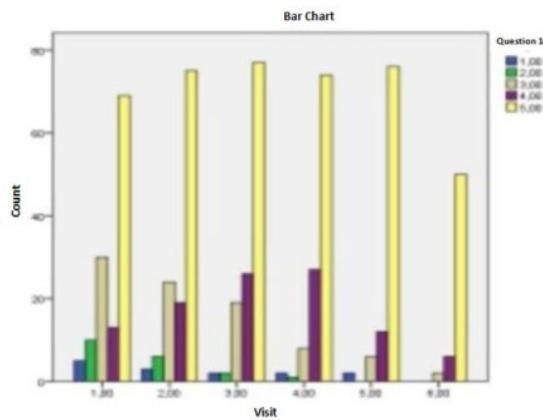


Figure 2. Visual inspection of teat and udder base soiling during visits.

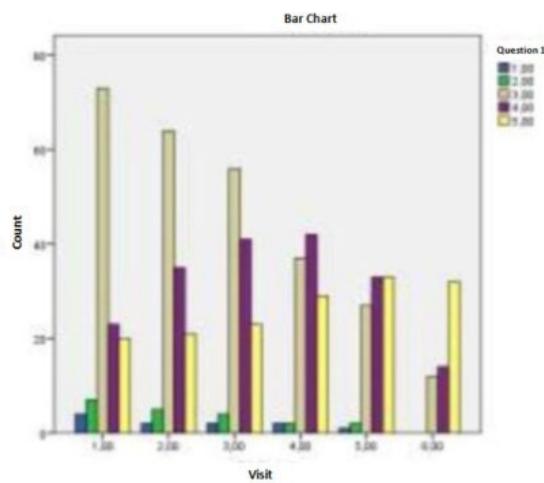


Figure 3. Maintenance of the cleanliness of the udder during visits.

Table 7 presents the results of univariate analysis of variance for TNM in milk in the conducted tests.

Table 7 shows that capacity, rearing system of cows, breed, number of cows in the facility and number of cows milked had a very significant effect on TNM. The factors of capacity occupancy, daily milk production and daily milk production per cow did not have a statistically significant impact.

The partial  $\eta^2$  test shows that the percentage of milk protein was most influenced by daily milk production (14.8%), capacity occupancy (12.5%), daily milk production per cow (6.6%), number of cows milked (5.0%), capacity (4.0%), and number of cows in a facility (3.8%).

Table 7. Univariate analysis of variance for TNM.

Dependent variable	Tests of effects between factors and parameters					
	TNM					
Source	Sum of squares type III	df	Average square	F	Significance	$\eta^2$
Corrected model	539.283 <sup>a</sup>	200	2.696	1.620	0.000	0.421
Section	31.387	1	31.387	18.863	0.000	0.041
Capacity	30.925	14	2.209	1.328	0.187	0.040
Rearing system	3.352	1	3.352	2.015	0.156	0.005
Breed	1.577	1	1.577	0.948	0.331	0.002
Milking system	1.167	1	1.167	0.701	0.403	0.002
No. of cows in the facility	29.029	10	2.903	1.745	0.069	0.038
Capacity utilization	105.594	32	3.300	1.983	0.001	0.125
No. of cows milked	39.288	16	2.456	1.476	0.104	0.050
Daily milk production	128.588	75	1.715	1.030	0.416	0.148
Daily milk production per cow	52.576	25	2.103	1.264	0.179	0.066
Error	740.457	445	1.664			
Total	8772.000	646				
Total corrected	1279.740	645				

a.  $R^2 = 0.421$  (Adjusted  $R^2 = 0.161$ ).

It should be taken into account that the most pronounced increase in scores related to TNM in milk was found between the 3<sup>rd</sup> and 4<sup>th</sup> visits and that the ANOVA test revealed significant differences between visits ( $F=9.63$ ,  $P<0.0001$ ), and by using the LSD test, very significant differences were found between the 1<sup>st</sup> and 4<sup>th</sup>, 1<sup>st</sup> and 5<sup>th</sup> and 1<sup>st</sup> and 6<sup>th</sup> visits. Using the  $\chi^2$  test, very significant differences in TNM were found between visits. In essence, the number of scores 4 and 5 was found to increase in relation to the number of visits, especially for the 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> visits, which should be taken into account when defining good hygiene practices.

Table 8 shows a univariate analysis of variance for the results related to SCC in milk in the conducted tests. A gradual increase was observed in the mean values of SCC from the first to the third visit, then a more pronounced increase between the third and fourth visits, and a slightly less pronounced increase at the fifth visit compared to the fourth. Some decrease in this parameter was observed between the fifth and sixth visits. Furthermore, significant differences between visits were found in the analysis of variance for SCC ( $F=5.17$ ,  $P<0.0001$ ). In addition, SCC values varied depending on the visit and the application of control measures. Using the LSD test, a very significant difference was found between the 1<sup>st</sup> and 5<sup>th</sup> visits and significant differences were found between the 1<sup>st</sup> and 4<sup>th</sup>, and 1<sup>st</sup> and 6<sup>th</sup> visits, as well as a very significant difference between the 2<sup>nd</sup> and 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup>, and between the 3<sup>rd</sup> and 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> visits, respectively. Finally, using the  $\chi^2$  test,

very significant differences were found in SCC values between visits. It was found that the rate of scores 4 and 5 increased in relation to the ordinal number of visits, especially at the 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> visits.

Table 8. Univariate analysis of variance for SCC.

Dependent variable	Tests of effects between factors and parameters						
	SCC	Sum of squares type III	df	Average square	F	Significance	$\eta^2$
Source							
Corrected model	403.831 <sup>a</sup>	200	2.019	1.460	0.001	0.396	
Section	34.137	1	34.137	24.691	0.000	0.053	
Capacity	32.294	14	2.307	1.668	0.059	0.050	
Rearing system	4.639	1	4.639	3.355	0.068	0.007	
Breed	2.772	1	2.772	2.005	0.157	0.004	
Milking system	1.425	1	1.425	1.030	0.311	0.002	
No. of cows in the facility	40.129	10	4.013	2.903	0.002	0.061	
Capacity utilization	101.318	32	3.166	2.290	0.000	0.141	
No. of cows milked	21.476	16	1.342	0.971	0.488	0.034	
Daily milk production	138.103	75	1.841	1.332	0.043	0.183	
Daily milk production per cow	28.332	25	1.133	0.820	0.718	0.044	
Error	615.227	445	1.383				
Total	10756.000	646					
Total corrected	1019.059	645					

a.  $R^2 = 0.396$  ( $R^2 = 0.125$ ).

All of the above results should be taken into account when determining farm and hygiene parameters that influence the increase in the SCC mean values. These parameters should be included in the consideration of corrective and preventive measures within the framework of good hygiene practices. It should also be taken into account that the analysis of the determined values shows that the number of visits had an influence on the percentage of milk fat ( $p<0.1$ ,  $F = 2.221$ ), while there was no influence on the protein percentage.

According to the research results, it is clear that certain hygiene procedures were of great importance before milking cows, such as visual examination of the udder for the presence of signs of inflammation or damage, visual examination of the teats and the base of the udder for their soiling, application of the pre-milking test, washing and disinfecting of teats and udders, application of the mastitis test, teat wiping procedures, udder massages, hygiene procedures before and during the installation of milking units, control of the milking machine, automatic vacuum interruption, total duration of milking from the beginning to the end of contact with the milker, as well as interruption of milking (poor actions of the milking personnel, noise, other cows, other animals, etc.), as determined by other authors

(Hristov et al., 1997; Tamime, 2009; Lemma et al., 2018). Furthermore, the research found that the cleanliness of the cow's body, a visual inspection of the teats and the base of the udders for their soiling by the milker, the overall cleanliness of the teats and trimming the hair from the udders had an impact on milk quality. Hygienic procedures after milking include the application of various disinfectants as soon as possible after removing the teat cups by dipping or spraying the teats (Hristov et al., 1997). The importance of maintaining the hygiene of the materials for wiping the udder and the accessories for the application of disinfectants was also highlighted. It has been stated that numerous studies show that the occurrence of new udder infections is directly related to greater soiling of the udder (Stewart et al., 2002; Berge and Baars, 2020). It is necessary to disinfect the teats of the udder immediately after milking with an agent that has the ability to close the teat opening. In addition, post-milking udder disinfection should be done regularly after each milking on farms, which is not the case on small farms where it is mostly poorly performed (Hristov et al., 1997; Hristov, 2002; Tamime, 2009).

Presently, there are several programs addressing the best management practices in milk production, including good hygiene practices (Costa et al., 2005), emphasizing that the key factor for milk quality is avoiding contamination of raw milk and dairy products. The failure to maintain adequate sanitary practices contributes to the bacterial contamination of milk, chemical substances or physical deterioration from various sources. Common predisposing factors for milk contamination include the milking environment, cow body, milking personnel, milking equipment, milk transport, utensils and liquids used during milking, which has been unequivocally confirmed in research. Finally, it is crucial to apply good hygienic practices in milk production, regardless of the size of the farm, to effectively protect public health in accordance with the claims made by Bekuma and Galmessa (2018).

European Union regulations require raw milk to come from animals without any symptoms of infectious diseases transmitted to humans through milk. The cows should be in good health and free of udder wounds, both of which could affect the quality of the milk. It is envisaged that milk from cows treated with approved medicinal products should be separated from milk originating from healthy cows (European Commission, 2004a). Therefore, animal health management aims to achieve and maintain a disease-free herd (Hillerton, 2004). This can be achieved when infected animals are cured or removed from the herd, and new infections are prevented. A closed herd production, when there is no introduction of animals from other farms, is an important measure for maintaining a disease-free herd. Treatment and separation of infected animals from the rest of the herd prevent the transmission of pathogenic microorganisms from cow to cow (Hillerton, 2004), as well as high-quality feed, facility hygiene and hygienic milking conditions. Mastitis control is an important issue for the dairy sector, so

mastitis control programs have been developed and implemented in many countries (Ekman et al., 2005; Olde Riekerink et al., 2005; Van der Zwaag, 2005). They are usually based on five crucial principles: disinfection after milking, antibiotic therapy for dry cows, appropriate treatment of clinical cases, removal of chronically infected cows and regular maintenance of milking machines (Akam et al., 1989). In Norway, in 1982, a successful udder health program was implemented, with the main focus of this program on milking and the repair of milking machines, although treatment of cows' udders by drying and dipping teats in disinfectant was not required to the same extent. Nevertheless, this, combined with a change in farmer attitudes and cow breeding programs, led to a 50% reduction in clinical mastitis treatment, a reduction in SCC (somatic cell count – an indicator of subclinical mastitis) from 250,000 to 114,000 in 1 mL of milk, and a significant reduction in treatment costs during ten years since 1994 (Østerås and Sølverød, 2005). Contemporary mastitis control programs anticipate limiting the frequency of mastitis, and thus reducing SCC in milk to an economically tolerable level is mainly achieved by applying strict hygienic and sanitary measures in milking parlors and barns. Limiting the frequency of infections in the udder quarters of cows involves teat disinfection after each milking, service and maintenance of the milking machine, back-washing of the milking cups and washing of the udders with running water before milking, as well as the application of other hygienic and sanitary measures (Hristov, 2002).

### Conclusion

Based on the results of the conducted study, it can be concluded that farm characteristics (capacity, housing system, breed, milking system, number of cows, capacity occupancy mostly had a very significant or significant impact on all hygiene parameters classified in the aforementioned categories, as well as on protein and fat content, SCC and TNM in milk. Very significant variations were found in the listed hygiene parameters of the farm depending on the farm visits, as well as a continuous trend of improving the scores of hygiene parameters of the farm in relation to the regular number of farm visits. Based on the obtained results, an increase in the mean values of the TNM scores was observed after each visit. The TNM ANOVA test revealed significant differences between visits ( $F = 9.63$ ,  $P < 0.0001$ ). For SCC mean values, an increase in scores was found from the first to the third visit, then a more pronounced increase between the third and fourth visits, and a slightly less pronounced increase in the fifth visit. Some decrease was observed for this parameter between the fifth and sixth visits. Analysis of variance for SCC revealed highly significant differences between visits ( $F = 5.17$ ,  $P < 0.0001$ ). The SCC significantly varied across all visits and control measures applied. Highly significant or significant differences were found between all visits

by the LSD test and  $\chi^2$  test. It was observed that the number of visits tended to have a significant influence on the milk fat rate ( $F=2.221$ ;  $P<0.1$ ), but no influence on the milk protein rate ( $P = 0.480$ ;  $F = 0.901$ ).

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## UTICAJ POBOLJŠANJA HIGIJENE TELA I VIMENA NA BEZBEDNOST I SASTAV MLEKA NA MALIM FARMAMA U SRBIJI

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### R e z i m e

Higijensko stanje muznih krava i njihovih vimena na 128 malih gazdinstava lociranih u različitim regionima Srbije, pretežno angažovanih na proizvodnji mleka, 5–15 krava, laktofriza i najmanje jedne mašine za mužu, procenjeno je korišćenjem sledećih indikatora: 1. opšta procena čistoće tela, 2. vizuelni pregled sisa i osnove vimena, i 3. održavanje čistoće vimena, ocenjeno na skali od 0 do 1 ili od 1 do 5. Uzorci su uzeti nakon hlađenja pri svakoj poseti farmi i testirani na sadržaj mlečnih proteina i masti, broj somatskih ćelija (engl. *somatic cell count* – SCC) i ukupan broj mikroorganizama (engl. *total number of microorganisms* – TNM). Sadržaj mlečne masti određen je Gerberovom metodom, sadržaj proteina Kjeldahlovom metodom, ukupan broj mikroorganizama metodom ISO 4833-1:2013, a broj somatskih ćelija upotreboom aparata Fossomatic TM. Program IBM SPSS je korišćen za statističku obradu podataka. Kapacitet, uslovi smeštaja, rasa, sistem muže, broj krava i popunjenoš kapaciteta su uglavnom imali veoma značajan ili značajan uticaj na higijenske parametre, sadržaj proteina i masti, broj somatskih ćelija i ukupan broj mikroorganizama u mleku. Higijenski parametri su imali kontinuirani trend poboljšanja u odnosu na posete. Srednje vrednosti ocena za ukupan broj mikroorganizama su se povećavale posle svake posete, uz značajne razlike između poseta ( $F=9,63$ ,  $P<0,0001$ ). Ocene broja somatskih ćelija su veoma značajno varirale između poseta ( $F=5,17$ ,  $P<0,0001$ ). Broj poseta je pokazao tendenciju značajnog uticaja na masnoću mleka ( $F=2,221$ ;  $P<0,1$ ), ali ne i na ideo proteina ( $P=0,480$ ;  $F=0,901$ ).

**Ključne reči:** unapređenje, telo, vime, higijena, mleko, bezbednost, sastav, mala farma.

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Materijal i metode treba izložiti jasno uz objašnjenje svih primenjenih postupaka u radu. Opšte poznate metode izložiti kratko, a detaljnije ih objasniti ukoliko se odstupa od ranije objavljenih postupaka. Za radove eksperimentalnog karaktera obavezno navesti način statističke obrade podataka. U ovom poglavlju, kao i u poglavlju „Rezultati i diskusija“, po potrebi se mogu dati i određena podpoglavlja.

### **Rezultati i diskusija**

U poglavlju „Rezultati i diskusija“ interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

### **Zaključak**

U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabranje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

### **Zahvalnica**

Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

### **Literatura**

Poglavlje „Literatura“ treba da sadrži samo radove citirane u glavnom tekstu. Rad citiran u tekstu treba da sadrži prezime autora i godinu. Ako citat obuhvata jednog autora on se navodi kao Jalikop (2010) ili (Jalikop, 2010). Kada citat obuhvata dva autora on se navodi kao Sadras i Soar (2009) ili (Sadras i Soar, 2009). Ako se u tekstu citiraju više od dva autora posle prezimena prvog autora navodi se skraćenica „et al.“, a zatim godina. Ovakav citat navodi se kao Lehrer et al. (2008) ili (Lehrer et al., 2008). Ako se za određeni problem istovremeno citira više radova onda se oni hronološki nabrajaju. Odvajanje većeg broja citiranih radova van

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Primeri navođenja referenci su sledeći:

#### **Periodičan časopis**

Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (*Helianthus annuus* L.). *Journal of Agricultural Sciences*, 54, 1-10.

#### **Knjiga**

Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

#### **Poglavlje u knjizi**

Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits*. (pp. 441-514). New York: John Wiley and Sons, Inc.

#### **Zbornik**

Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

#### **Teza**

Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

### **Izveštaj**

Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

### **Veb sajt**

Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from <http://research.amnh.org/entomology/spiders/catalog/index.html>

### **Rezime**

Rezime na srpskom jeziku (za rade napisane na engleskom jeziku) ili na engleskom jeziku (za rade napisane na srpskom jeziku) navodi se na kraju rada i treba da ima od 200 do 250 reči. Ispred osnovnog teksta rezimea, navodi se naslov rada, puno ime, srednje slovo i prezime svih autora i naziv i adresa ustanove autora. Naslov „Rezime“ piše se razmagnuto i centrirano. Nakon naslova sledi jedan razmak, a zatim tekst rezimea, uvučen jednim tabulatorom. Neposredno nakon teksta rezimea, navode se ključne reči, sa tačkom na kraju. E-mail adresa autora za kontakt navodi se ispod crte, pri dnu stranice.

### **Tabele**

Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

### **Ilustracije**

Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika“ (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF“ ili „JPG“ formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

### **Skraćenice i jedinice**

U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod

uslovom da se ti izrazi navedu u punom obliku prilikom prvog pominjanja, sa skraćenim oblikom u zagradi. Vrednosti od 1 do 9 mogu se izražavati slovima, a ostali brojevi isključivo numerički.

### **Nomenklatura**

Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* itd.

### **Formule**

Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation“. Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

Nakon objavlјivanja rada, autoru za kontakt će biti poslat jedan primerak časopisa. Mole se svi budući saradnici da rad pripreme prema datom uputstvu, kako bi olakšali rad redakcije časopisa. Ukoliko se rad ne pripremi po navedenom uputstvu neće biti prihvaćen za objavlјivanje.

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