



IMPACT OF HERBAL BLENDS SUPPLEMENTATION ON THE MINERAL PROFILE OF BLOOD PLASMA AND MILK OF DAIRY COWS*

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Abstract

The present study aimed to evaluate the effect of supplementation of cow's diet with four different herbal blends on the mineral profile in the plasma and milk of cows in the first stage of lactation. The experiment involved 50 cows divided into five groups. Four herbal blends were used as an experimental factor. A full ration of 100 g of the herbal supplements per 100 kg b.w. per day was included in the cow's diet from one week before the planned calving to the end of the third month of lactation. The feed intake by the dairy cows was assessed in three 3-day cycles in lactation weeks 6, 7, and 8. Concurrently, blood and milk were collected from these cows. The content of selected minerals was determined in the feed rations, herbal blends, milk, and plasma. The study showed that herbs, besides being a source of bioactive compounds, should be considered a beneficial source of minerals, especially microelements like copper, zinc and iron, and might affect the mineral homeostasis in the organism. The introduction of different herbal blends affected not only the mineral profile of blood but most notably the milk mineral profile.

Key words: dairy cows, herbs, minerals, milk, blood plasma

The nutrition of high-yielding dairy cows must be balanced not only in terms of energy and protein but also concerning essential macro- and micronutrients (Seyfaddinov, 2022). An inadequate supply of minerals can lead to metabolic disorders, which remain a significant health challenge despite careful dietary management (Puppel and Kuczyńska, 2016; Harvey et al., 2021). Ruminants have a specific regulation of mineral homeostasis that enables them to cope with nutrient fluctuations (Wilkins and Muscher-Banse, 2020). However, during the transition period, decreased appetite and increased nutrient excretion due to intensive lactation can cause imbalances in metabolism, affecting vitamins and trace elements in blood plasma (Goff, 2018; Abo El-Maaty et al., 2021). These elements are vital for physiological functions, regulating metabolic processes and supporting health, immune and endocrine systems, growth, and reproduction (Yaremchuk and Farionik, 2022; Fadlalla et al., 2020; Harvey et al., 2021). The degree of utilization of elements in the animal organism is determined by the correlation between the amount and type of nutrients and minerals contained in feeds, the condition of the intestinal epithelium, and the overall health of the animals.

Recently, there has been increased scientific interest in the use of feed additives to alleviate mineral disorders in dairy cows (Yazlık et al., 2021; Del Río-Avilés et al., 2022). As the milk potential of cows increases, more attention is being paid to their role as cofactors in the various biochemical processes involved in lactogenesis, as this affects the overall quality and quantity of milk secreted (Erickson and Kalscheur, 2020; López-Alonso and Miranda, 2020).

Properly selected feed additives, including mineral-rich herbs and bioactive substances, such as polyphenols and essential oils, with targeted effects, can support the cow body's adaptation to the new period and contribute to reducing health problems (Aleri et al., 2016; Tamminen et al., 2018; Redoy et al., 2020). They stabilize the changes taking place in the rumen and the liver (Rivera-Chacon et al., 2022), modify the intestinal microbiota (Xie et al., 2020) contribute to stimulation of feed intake and utilization (AlSuwaiegh et al., 2022), and indirectly contribute to better health status (Sharma et al., 2021), immunity (Shan et al., 2018), and higher nutritional value of milk (Cui et al., 2021). Furthermore, the use of phytobiotics in the form of herbs can also have an

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anabolic effect, promoting the processes of tissue growth and development, resulting in better performance and production (Kikusato, 2021). However, there is still little information about the effect of herbal blends on the level of minerals in blood plasma and milk from cows. Due to the different physiological processes occurring in the digestive systems of monogastric and ruminant animals, herbs used in the nutrition of ruminants may exhibit different properties compared to their original action and use in monogastric animals (Kiczorowska et al., 2017; Mirowski, 2019; Wójtowski et al., 2023). Especially, the absorption of minerals can be significantly influenced by various herbs, particularly those rich in polyphenols, which may either enhance or inhibit their bioavailability (Muttepawar et al., 2014). Also, the use of complex herbal mixtures has been proven to provide better results in treating specific problems than using single herbal components (Hashemzadeh-Cigari et al., 2014).

Therefore, it was hypothesized that due to the multi-directional properties of herbs and their possible synergistic effect, dedicated herbal additives to cow feed rations, through the contribution of mineral and bioactive components, can modulate the processes of lactogenesis and improve milk quality in high-yielding cows and contribute to the improvement of animal health. This impact was assessed by determining the effects of four different herbal mixtures, as a source of minerals and bioactive components, on the mineral profile of plasma and milk in cows in the early stage of lactation.

Material and methods

Experimental animals and management

The research was conducted with the consent of the Local Ethics Committee on Animal Experimentation of the University of Life Sciences in Lublin.

The study was conducted on a private farm on a herd of 180 Polish Holstein-Friesian cows of Black-and-White variety. The average annual milk yield was 9870 kg per cow. Cows were kept in a tie-stall barn. To provide a full intake of experimental additives by each

individual, the PMR feeding system was used. The feed rations for the cows were designed according to the actual nutritional value of feeds assessed by chemical analyses in compliance with the INRA (2019) ruminant feeding system (Strzetelski, 2014). The year-round rations for the cows included a basic partially mixed ration (ensuring the production of 28 l of milk per cow) composed of maize silage, grass haylage, meadow hay, and concentrate mixture (Table 2). Additionally, the cows received the concentrated mixture according to the current nutritional requirement of each individual. The body condition score (BCS) of each experimental cow was evaluated using a binary system that considered a change of at least 0.5 points on the body condition score (BCS) scale between consecutive measurements.

The experiment used 50 dairy cows involved in the study based on analogs (taking into account age: 3rd lactation; milk yield from previous lactation: 7790 to 7910 kg; physiological period: end of pregnancy). The cows selected for the experimental groups exhibited similar body weight and health status. The average BCS of cows from both the control and experimental groups during the periparturient period was 3.0. Based on our own analysis of the available literature, our preliminary research (Klebaniuk et al., 2014, 2017; Fabjanowska et al., 2024) and the knowledge gained so far, the following herbal raw materials were selected to compose 4 herbal mixtures (D1, D2, D3, D4; Table 1): thyme (*Thymus vulgaris* L.), purple coneflower (*Echinacea purpurea*), oregano (*Origanum vulgare*), stinging nettle (*Urtica dioica*), chamomile (*Matricaria chamomilla*), garlic cloves (*Allium sativum* L.), sage leaf (*Salvia officinalis* L.), rosemary leaf (*Salvia rosmarinus*), cinnamon tree bark (*Cinnamomum* Scheffer) and cumin (*Cuminum cyminum*). All raw materials have a documented antioxidant, antibacterial, and immunomodulatory effect (Kumar et al., 2014; Ayrle et al., 2015; Bhatt, 2015; Ezzat Abd El-Hack et al., 2016; Abo El-Maaty et al., 2021; Kuralkar and Kuralkar, 2021). The animals were selected successively from the herd at the onset of the dry period. Experimental cows were divided into five research groups (n=10 animals each): control (C) and four experimental ones: D1, D2, D3, and D4 (Table 1).

Table 1. Experimental design

Nutrition		Group				
		Control	Experimental			
		C (n=10)	D1 (n=10)	D2 (n=10)	D3 (n=10)	D4 (n=10)
Roughage		+	+	+	+	+
Concentrated feed		+	+	+	+	+
Experimental factor (100 g/100 kg body weight/day)						
Herbal blend	D1	–	+	–	–	–
	D2	–	–	+	–	–
	D3	–	–	–	+	–
	D4	–	–	–	–	+

D1 – herbal blend containing: thyme *Thymus vulgaris* L., purple coneflower *Echinacea purpurea* (L.) Moench, oregano *Origanum vulgare* L., and cinnamon *Cinnamomum verum* J. Presl; D2 – thyme *Thymus vulgaris* L., purple coneflower *Echinacea purpurea* (L.) Moench, garlic *Allium sativum* L., common nettle *Urtica dioica* L., and caraway *Carum carvi* L.; D3 – thyme *Thymus vulgaris* L., purple coneflower *Echinacea purpurea* (L.) Moench, oregano *Origanum vulgare* L., common nettle *Urtica dioica* L., caraway *Carum carvi* L., sage *Salvia officinalis* L., and chamomile *Matricaria chamomilla* L.; D4 – thyme *Thymus vulgaris* L., purple coneflower *Echinacea purpurea* (L.) Moench, oregano *Origanum vulgare* L., garlic *Allium sativum* L., common nettle *Urtica dioica* L., sage *Salvia officinalis* L., and chamomile *Matricaria chamomilla* L.

Table 2. Chemical composition and nutritional value of feeds, concentrated mixture, and experimental herbal blends

Component	MH	CM	MS	GH	D1	D2	D3	D4
Dry matter (DM) (%)	90.3	86.7	35.7	39.2	89.3	89.3	89.6	89.4
per kg DM								
Crude protein (g)	127.8	186.7	75.2	112.7	156.8	186.0	174.2	179.3
Crude fiber (g)	262.1	66.8	209.4	359.2	206.8	193.6	196.7	198.3
Ether extract (g)	14.8	14.2	37.2	13.1	25.0	24.0	23.8	24.2
NFC (g)	543.6	679.6	646.1	458.4	553.6	542.2	546.4	539.6
UFL	0.80	1.13	0.90	0.76	0.87	0.86	0.87	0.87
PDIE (g)	75	108	74	68	95	106	98	99
PDIN (g)	68	124	53	76	90	118	114	115
LFU	1.05	0	1.12	1.23	0.96	0.97	0.97	0.96
Potassium (g)	19.04	8.75	10.90	31.70	10.48	11.94	11.23	11.83
Sodium (g)	0.14	5.08	0.11	0.20	0.53	1.43	0.51	0.44
Calcium (g)	4.43	13.27	3.19	7.90	16.56	11.87	13.50	15.93
Magnesium (g)	1.58	4.38	1.48	2.35	3.65	4.02	3.37	4.74
Phosphorus (g)	2.38	6.92	2.62	3.30	1.20	2.77	3.37	3.65
Copper (mg)	2.25	20.01	7.02	6.50	6.11	35.55	8.14	22.03
Manganese (mg)	103.94	143.40	23.70	90.10	129.70	71.29	175.66	299.74
Iron (mg)	136.40	135.49	234.20	207.50	252.40	632.85	615.18	862.45
Zinc (mg)	21.13	147.34	29.40	27.20	28.15	28.83	40.23	49.43
TP (mg)					2118.80	2321.06	2227.54	2786.87

MH – meadow hay; MS – maize silage; GH – grass haylage; CM – concentrated mixture; D1, D2, D3, D4 – experimental herbal blends; NFC – non-fibrous carbohydrates; UFL – unit for milk production; LFU – filling unit of roughage for dairy cows; PDIE – protein digested in the intestine when rumen fermentable energy is limiting; PDIN – protein digested in the intestine when rumen fermentable nitrogen is limiting; TP – average polyphenol content, mg GAE/kg DM.

Table 3. Composition of concentrate mixture and herbal blends administered to the cows during the experiment, %

Component	Concentrated mixture	Herbal blend			
	CM	D1	D2	D3	D4
Barley	19.5				
Wheat	18.0				
Maize	20.0				
Bran	8.0				
Protein supplement blend (35% protein)	32.0				
Vitamin and mineral blend ¹	2.5				
Thyme, <i>Thymus vulgaris</i> L.		25	15	20	20
Purple coneflower, <i>Echinacea purpurea</i> L.		35	35	12	12
Oregano, <i>Origanum vulgare</i> L.				25	25
Garlic, <i>Allium sativum</i> L.			20		10
Common nettle, <i>Urtica dioica</i> L.			15	15	20
Caraway, <i>Carum carvi</i> L.			15	15	
Cinnamon, <i>Cinnamomum verum</i> J. Presl		15			
Sage, <i>Salvia officinalis</i> L.		25		8	8
Chamomile, <i>Matricaria chamomilla</i> L.				5	5

¹In 1 kg of the mix for lactating cows (Dolfos., Poland): vit. A 900 000 IU, vit. D₃ 400 000 IU, vit. E 7700 mg, vit. B₁ 100 mg, vit. B₂ 65 mg, vit. B₆ 50 mg, vit. B₁₂ 400 µg, pantothenic acid 150 mg, folic acid 12 mg, choline 100 mg, biotin 200 000 µg, calcium 170 g, phosphorus 15 g, sodium 100 g, magnesium 50 g, zinc 7000 mg, sulfur 10.8 g, manganese 9000 mg, copper 2000 mg, cobalt 23 mg, iodine 150 mg, selenium 50 mg; the same amount of the premix was given to each group of cows.

Four herbal blends: D1, D2, D3, and D4 were used as the experimental factor (Table 3). The additives were introduced into the feed rations for the cows gradually. The herbal supplements (beginning from 50 g to the full ration of 100 g per 100 kg b.w. per day) was administered to the cows from one week before the planned calving to the end of the 8th week of lactation. The blends were given with the concentrated mixture (CM). The cows were fed three times a day. The feed rations administered after calving were designed based on the yield predicted from data on the previous lactation. Next, as the yield increased, the rations were adjusted every 7 days by the addition of the concentrated mixture at a level of 0.45 UMP per kg of milk.

Sampling and chemical analyses

The feed intake by the dairy cows was assessed in three 3-day cycles at 4, 6, and 8 weeks of lactation. The supplied feed and leftovers were weighed (approximately an hour before the next feeding) to determine the feed intake. The collected samples of feeds were dried, and then the samples were ground to obtain a uniform material that was representative of the analyzed feed. The feed and the experimental herbal blends were analyzed to determine the content of basic nutrients, i.e. dry matter, total protein, crude fat, and crude fiber, according to the AOAC analytical procedures (AOAC, 2011). The nutritional value of the feeds and doses was estimated using a computer program WINWAR, version 1 (WINWAR 2.1.3.13. 2007).

Blood and milk were sampled in 8th week of lactation. Blood was collected from the external jugular vein into heparin tubes in the morning after milking and before feeding. Milk samples were collected into disposable containers during morning milking. The blood and milk samples were cooled to a temperature of approx. 4°C and transferred directly for laboratory analyses.

The content of the selected minerals in the feeds and milk was determined with the AAS atomic absorption spectrophotometry method on the UNICAM 939/959 apparatus following the principles of chemical analysis of CLB UP in Lublin. The total phosphorus content was determined as in the Fiske and Subbarow method (1926).

Total polyphenol content was analyzed in the experimental mixtures (D1, D2, D3, D4). In this process, 10 grams of each herbal mixture were mixed with 90 mL of distilled water and heated in a water bath at 40°C for 10 hours, following the procedure outlined by Kozłowska et al. (2015). After heating, the mixture was filtered through filter paper to separate the solid residues from the liquid. The freshly obtained aqueous extracts were subsequently used to analyze the total polyphenol content according to the procedure described by Kawęcka et al., (2022). In summary, the Singleton and Rossi method with the Folin-Ciocalteu reagent was used in the analysis (Singleton and Rossi, 1965). A 0.1 ml sample was combined with the reagent and 20% (w/w) sodium carbonate and incubated for 30 minutes. The absorbance of the mixture was re-

corded at 700 nm using a Thermo Fisher Scientific spectrophotometer (UV-Visible Evolution™ 60S spectrophotometer). Gallic acid, in the concentration range of 1–150 mg/L, was used as a standard. Results were expressed as gallic acid equivalents (GAE) in mg per gram of sample DM.

Plasma for the analysis of biochemical parameters was obtained by centrifugation of whole blood at 3000 rpm for 10 min in a laboratory centrifuge MPW350R (producer: MPW Medical Instruments, Warsaw, Poland) at a temperature of 4°C. After centrifugation, plasma potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), phosphorus (P), zinc (Zn), copper (Cu), and iron (Fe) levels were determined using reagent kits (BioMaxima, Lublin, Poland) according to the manufacturer's protocols and a random access biochemical analyzer Metrolab 2300 GL (producer: Metrolab SA, Buenos Aires, Argentina).

Statistical analyses

The data were analyzed with one-way analysis of variance ANOVA ($\alpha=95$, $P<0.05$) using Statistica software version 13.3.721.0 (StatSoft Poland Sp. z o.o., Kraków, Poland, 2022, www.statsoft.pl) (accessed on 7 June 2024) with an individual animal as a statistical unit. The Shapiro-Wilk and Brown-Forsythe tests were used for testing the normality of the data and homogeneity of variances, respectively. The mathematical model used for the analysis was:

$$Y_{ij} = \mu + a_i + e_{ij},$$

where Y_{ij} is the measured variable, μ is an overall mean, a_i is the effect of the treatment, and e_{ij} is the random error. Significant differences between the means were determined by Tukey's honestly significant difference (HSD) post hoc test.

Additionally, to check the correlations between the herbal blend addition to the feed rations and the mineral profile in the blood plasma and milk, Pearson's correlation coefficients (r) were calculated.

Results

Both the chemical composition and the nutritional value of the feeds administered to the cows during the study were typical and did not differ from the values reported in the literature (Strzetelski et al., 2014; INRA, 2019; NASEM, 2021). The experimental cows received both roughage and concentrated feed in compliance with the requirement guidelines. The feed and the basic nutrient intake did not differ significantly between the control (C) and experimental treatments (D1, D2, D3, and D4) (Table 4), with the highest feed intake in the D2 and D3 groups. However, on the 100th day of lactation, the average BCS in the control group was 3.25, while in the experimental groups, the average BCS was 3.5 ($P=0.044$).

The analysis of the total polyphenol content of blends D1, D2, D3 and D4 showed that the average polyphenol content of the samples tested was 2363.52 mg GAE/kg DM. The highest polyphenol content was recorded in blend D4 (Table 2).

Analysis of mineral element content in the herbal mixtures showed slight differences in the content of mac-

roelement levels in the diets of the experimental cows (Table 4). In particular, groups D2, D3 and D4 received the highest content of key macroelements such as potassium and calcium. Calcium levels were higher in the herbal-supplemented groups (D1–D4) compared to the control group (C). However, the content of microminerals was notably affected by the addition of herbs.

Table 4. Daily intake of feed and nutrients by cows

Indicator		Group*					
		C	D1	D2	D3	D4	
kg/day							
Meadow hay	MH	3.8	3.8	3.8	3.8	3.8	
Concentrated mixture	CF	7	7	7	7	7	
Maize silage	MS	30	30	30	30	30	
Grass haylage	GH	5	5	5	5	5	
Herbal blend	D1		0.7				
	D2			0.7			
	D3				0.7		
	D4					0.7	
Intake per feed ration							P-value
UFL		20.73	20.3	21.26	21.35	20.84	0.186
PDIE (g)		1839	1863	1894	1890	1876	0.209
PDIN (g)		1702	1749	1816	1821	1807	0.089
LFU		18	18.2	17.9	18	18.3	0.306
DM intake (kg)		22.2	22.9	23.2	23.1	22.9	0.138
Potassium (g)		297.31	303.86	304.77	304.35	304.71	0.167
Sodium (g)		32.88	33.21	33.77	33.20	33.16	0.216
Calcium (g)		145.39 b	155.74 a	152.81 ab	153.85 ab	155.35 a	0.037
Magnesium (g)		52.46	54.74	54.97	54.57	55.43	0.128
Phosphorus (g)		84.69	85.44	86.42	86.81	86.98	0.146
Copper (mg)		217.09 c	220.90 b	239.31 a	222.19 b	230.87 a	0.027
Manganese (mg)		1657.38	1738.45	1701.94	1767.55	1844.95	0.234
Iron (mg)		4205.31 b	4363.09 b	4600.91 a	4591.15 a	4745.03 a	0.018
Zinc (mg)		1334.90 b	1352.49 ab	1352.92 ab	1360.13 a	1365.83 a	0.045

a, b – values marked with different letters differ significantly between the groups at $P < 0.05$; C – cows receiving no herbal blend supplementation; D1 – cows receiving feed supplemented with herbal blend D1: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Origanum vulgare* L., and *Cinnamomum verum* J. Presl; D2 – cows receiving feed supplemented with herbal blend D2: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Allium sativum* L., *Urtica dioica* L., and *Carum carvi* L.; D3 – cows receiving feed supplemented with herbal blend D3: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Origanum vulgare* L., *Urtica dioica* L., *Carum carvi* L., *Salvia officinalis* L., and *Matricaria chamomilla* L.; D4 – cows receiving feed supplemented with herbal blend D4: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Origanum vulgare* L., *Allium sativum* L., *Urtica dioica* L., *Salvia officinalis* L., and *Matricaria chamomilla* L.; MH – meadow hay; MS – maize silage; GH – grass haylage; CM – concentrated mixture; D1, D2, D3, D4 – experimental herbal blends; DM – dry matter; UFL – unit for milk production; LFU – filling unit of roughage for dairy cows; PDIE – protein digested in the intestine when rumen fermentable energy is limiting; PDIN – protein digested in the intestine when rumen fermentable nitrogen is limiting.

The content of minerals, both macro- and micronutrients, determined in the blood plasma were within the reference values characteristic for cattle (Winnicka, 2021) (Table 5). Significant differences in the level of the analyzed macroelements in the blood plasma of the experimental cows were found. The introduction of herbal supplementation to the nutrition of dairy cows contributed to an increase in the content of calcium ($C < D2 < D3 < D1 < D4$; $P = 0.018$) and magnesium ($C < D4 < D1 < D3 < D2$; $P = 0.039$) in the blood plasma of these animals. The highest effec-

tiveness in this respect was exhibited by the thyme- and nettle-containing herbal blends (D2, D3 and D4). Opposite results were obtained in the case of the sodium content. A lower level of this macronutrient was determined in the D2 and D4 experimental groups, and it was even by approximately 5% lower than in the control ($P = 0.047$). The level of potassium and phosphorus in the blood plasma of the cows in the C and D treatments was similarly stable, regardless of the experimental factors used ($P = 0.235$ and $P = 0.304$, respectively).

Table 5. Content of minerals in blood plasma

Indicator	Group										P-value
	C		D1		D2		D3		D4		
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	
mmol l ⁻¹											
Potassium	4.46	0.27	4.58	0.252	4.59	0.268	4.64	0.207	4.85	0.228	0.235
Sodium	143.9 a	14.87	148.0 a	12.62	136.4 b	18.51	145.6 a	10.72	136.5 b	14.53	0.047
Calcium	2.16 b	0.208	2.37 a	0.199	2.28 ab	0.274	2.29 ab	0.194	2.44 a	0.183	0.018
Magnesium	0.76 b	0.207	0.91 a	0.211	1.08 a	0.215	0.94 a	0.232	0.84 ab	0.238	0.039
Phosphorus	1.97	0.187	2.03	0.175	2.19	0.185	2.16	0.163	2.14	0.404	0.304
µmol l ⁻¹											
Copper	17.21 b	2.091	18.61 b	1.874	20.61 a	2.571	17.90 ab	1.968	18.80 a	2.467	0.026
Iron	23.74 b	2.874	28.40 ab	3.154	33.56 a	3.243	31.69 a	2.963	33.45 a	3.092	0.031
Zinc	15.75 b	2.215	16.36 ab	2.745	16.93 b	2.653	17.72 b	2.713	18.92 a	2.311	0.015

a, b – values marked with different letters differ significantly between the groups at P<0.05; C – cows receiving no herbal blend supplementation; D1 – cows receiving feed supplemented with herbal blend D1: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Origanum vulgare* L., and *Cinnamomum verum* J. Presl; D2 – cows receiving feed supplemented with herbal blend D2: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Allium sativum* L., *Urtica dioica* L., and *Carum carvi* L.; D3 – cows receiving feed supplemented with herbal blend D3: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Origanum vulgare* L., *Urtica dioica* L., *Carum carvi* L., *Salvia officinalis* L., and *Matricaria chamomilla* L.; D4 – cows receiving feed supplemented with herbal blend D4: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Origanum vulgare* L., *Allium sativum* L., *Urtica dioica* L., *Salvia officinalis* L., and *Matricaria chamomilla* L.

Table 6. Milk yield and chemical composition of milk of experimental cows

Indicator	Group										P-value
	C		D1		D2		D3		D4		
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	
Milk yield (kg)	36.5	4.21	37.6	3.21	40.7	5.16	39.5	4.02	38.4	4.76	0.066
Fat (%)	4.01	0.046	4.29	0.059	4.13	0.061	4.22	0.081	4.15	0.053	0.080
Protein (%)	3.12 b	0.148	3.29 ab	0.173	3.40 a	0.158	3.41 a	0.125	3.38 a	0.115	0.048
Lactose (%)	4.89	0.104	4.61	0.133	4.77	0.093	4.82	0.128	4.87	0.189	0.095
Bacteria count, CFU (10 ³)	12 a	0.951	11 a	1.283	9.4 b	1.010	10 b	1.227	9.5 b	1.080	0.044
Somatic cell count (10 ³)	225 a	31.7	153 ab	40.8	121 b	23.6	161 ab	35.7	126 b	27.7	0.015
g l ⁻¹											
Potassium	1.02	0.062	1.23	0.071	1.32	0.065	1.21	0.063	1.41	0.09	0.145
Sodium	0.37	0.031	0.26	0.028	0.19	0.025	0.27	0.024	0.29	0.052	0.231
Calcium	0.90 b	0.047	1.05 a	0.051	0.98 ab	0.043	0.94 b	0.038	1.09 a	0.071	0.041
Magnesium	0.09	0.011	0.11	0.01	0.14	0.012	0.09	0.01	0.10	0.009	0.118
Phosphorus	0.96	0.046	1.00	0.048	1.05	0.052	1.01	0.037	1.05	0.053	0.076
mg l ⁻¹											
Copper	0.33 b	0.019	0.37 b	0.021	0.48 a	0.021	0.47 a	0.02	0.46 a	0.018	0.023
Iron	0.27 b	0.061	0.33 b	0.089	0.58 a	0.131	0.42 ab	0.085	0.38 ab	0.057	0.034
Zinc	3.91 b	0.411	4.31 ab	0.721	4.51 ab	0.437	5.24 a	0.683	5.34 a	0.702	0.016

a, b – values marked with different letters differ significantly between the groups at P<0.05; C – cows receiving no herbal blend supplementation; D1 – cows receiving feed supplemented with herbal blend D1: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Origanum vulgare* L., and *Cinnamomum verum* J. Presl; D2 – cows receiving feed supplemented with herbal blend D2: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Allium sativum* L., *Urtica dioica* L., and *Carum carvi* L.; D3 – cows receiving feed supplemented with herbal blend D3: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Origanum vulgare* L., *Urtica dioica* L., *Carum carvi* L., *Salvia officinalis* L., and *Matricaria chamomilla* L.; D4 – cows receiving feed supplemented with herbal blend D4: *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, *Origanum vulgare* L., *Allium sativum* L., *Urtica dioica* L., *Salvia officinalis* L., and *Matricaria chamomilla* L.

The content of the investigated micronutrients in the plasma of the control and experimental animals varied significantly (Table 5). The supplementation of the feed with the herbal blends increased the content of copper

in the D2 and D4 treatments, on average by 20 and 9%, respectively (P=0.026). The use of these blends in the experimental groups also increased the plasma iron content (C<D1<D3<D4<D2; P=0.031). In the case of the zinc

level, a significant effect of the application of the herbal blend was noted only in the D4 treatment ($P=0.015$).

The analysis of the data in Table 6 revealed differences in the daily milk yield of the cows evaluated ($C<D1<D4<D3<D2$), however not confirmed statistically ($P\geq 0.05$). In terms of the content of the basic components, the milk of the cows receiving the D2 herbal blend contained on average 9% more total protein and 3% more fat, as compared to control milk, which resulted in a significantly ($P\leq 0.01$) improved protein-fat ratio, i.e. 0.82. In contrast, the D1 group was characterized by the lowest content of lactose. The inclusion of herbs, as an experimental factor, also reduced the count of somatic cells ($C>D3>D1>D4>D2$) and bacteria in milk ($C>D1>D3>D4>D2$), thus improving its safety.

In the macroelement profile in the milk of the experimental cows, calcium concentration was found to be the most prone ($P=0.041$) to be affected by herbal supplementation (Table 6). The addition of the D1 and D4 herbal blends to the dairy cow diets significantly increased the amount of this element in the milk, compared to the control group (by approximately 17% and 21% respectively; $C<D3<D2<D1<D4$). But, the most visible changes were noticed in the case of microelement content in milk. The copper content in the milk of D2, D3 and D4 cows was on average 42% higher ($P=0.023$) than in the

control group. The Fe content of the milk of the experimental cows showed a similar trend ($C<D1<D4<D3<D2$; $P=0.034$). In contrast, multidirectional changes were observed in zinc content in milk. Its level in D3 and D4 treatments was about 35% higher ($P=0.016$) than in the milk of non-supplemented animals.

The inclusion of herbs in the diet was significantly correlated with both analyzed traits. The values of Pearson's correlation coefficients (r) showed a strong or very strong significant impact of the amount of minerals contained in the diet with their concentration in milk (Tables 7–9). In particular, the amount of iron was affected by the content of this element in the diet – analysis showed an almost linear positive correlation ($r\sim 0.96$). Similarly, the content of calcium, potassium, copper, and zinc was very strongly positively correlated with the intake ($r\sim 0.8$). For other elements, like sodium, phosphorus, copper, or zinc the correlation coefficient values showed a strong ($r\sim 0.6$) relation. Similarly, in terms of the correlation between the intake of minerals and their concentration in blood plasma, a very strong or strong positive correlation was found in the case of calcium, copper, iron, and zinc. Whereas, analysis of minerals content in blood and milk showed a positive strong significant correlation only in the case of calcium, phosphorus, copper, and iron ($r\sim 0.6$).

Table 7. Pearson's correlation coefficients (r) between the intake of particular elements with feed ration and its concentration in cows' milk

Content in milk	Intake of elements with the diet							
	K	Na	Ca	Mg	P	Cu	Fe	Zn
K	0.812	0.604	0.142	0.213	0.388	0.152	0.194	0.178
Na	0.298	0.652	0.651	0.188	0.198	0.269	0.091	0.022
Ca	0.096	0.348	0.709	0.332	0.560	0.534	0.444	0.661
Mg	0.431	0.641	0.344	0.464	0.296	0.466	0.635	0.216
P	0.278	0.574	0.674	0.672	0.683	0.740	0.644	0.639
Cu	0.324	0.150	0.537	0.436	0.530	0.682	0.591	0.431
Fe	0.195	0.481	0.523	0.222	0.529	0.720	0.963	0.405
Zn	0.037	0.212	0.652	0.359	0.465	0.494	0.310	0.757

Values of correlation coefficients in bold are statistically significant ($P\leq 0.05$).

Table 8. Pearson's correlation coefficients (r) between the intake of particular elements with feed ration and its concentration in cows' blood plasma

Content in blood	Intake of elements with the diet							
	K	Na	Ca	Mg	P	Cu	Fe	Zn
K	0.223	-0.021	0.296	0.219	0.233	0.197	0.026	0.293
Na	0.128	0.285	0.053	0.151	0.021	0.121	0.237	-0.024
Ca	0.461	0.172	0.635	0.452	0.482	0.460	0.245	0.526
Mg	0.327	0.448	0.236	0.540	0.239	0.342	0.403	0.169
P	0.554	0.449	0.524	0.527	0.415	0.622	0.483	0.554
Cu	0.345	0.480	0.299	0.369	0.258	0.780	0.498	0.205
Fe	0.590	0.560	0.608	0.607	0.548	0.645	0.854	0.522
Zn	0.243	0.040	0.280	0.216	0.327	0.264	0.080	0.637

Values of correlation coefficients in bold are statistically significant ($P\leq 0.05$).

Table 9. Pearson's correlation coefficients (r) between the concentration of particular elements in cows' blood plasma and its concentration in cows' milk

Content in milk	Content in blood							
	K	Na	Ca	Mg	P	Cu	Fe	Zn
K	0.281	0.043	0.436	0.272	0.586	0.370	0.645	0.364
Na	0.128	0.198	0.269	0.273	0.554	0.173	0.501	0.221
Ca	0.281	0.062	0.552	0.099	0.618	0.222	0.538	0.175
Mg	0.045	0.102	0.136	0.166	0.321	0.491	0.439	0.000
P	0.177	0.023	0.583	0.158	0.534	0.341	0.447	0.229
Cu	0.048	0.224	0.075	0.367	0.361	0.671	0.260	0.066
Fe	0.073	0.149	0.287	0.365	0.482	0.503	0.650	0.189
Zn	0.330	-0.031	0.380	0.018	0.201	0.097	0.452	0.482

Values of correlation coefficients in bold are statistically significant ($P \leq 0.05$).

Discussion

Although, compared to the protein and energy content of the diet, minerals and trace elements have less impact on the overall performance of the animals they should not be considered less important due to their role in the regulation of the biological processes in organisms (Tuhy et al., 2018; Tsiamadis et al., 2016). The metabolic stress resulting also from an energy deficit in transition cows additionally affects the functioning of the immune system (Aleri et al., 2016; Fabjanowska et al., 2023). Therefore, the selection of appropriate feeding strategies, such as an adequate balance of mineral elements in the ration, is an effective strategy to promote and maintain an optimal immune status in dairy cows, which is crucial for health and directly contributes to maintaining high productivity in high-yielding cows (Daniel et al., 2020; Bates et al., 2022).

Among the active substances of herbs, the presence of polyphenols can variously affect the absorption of minerals. On the one hand, they form insoluble complexes with minerals and bind proteins, which limits their bioavailability and absorption in the gastrointestinal tract (Mascitelli and Goldstein, 2014; El-Saadony et al., 2024). The binding capacity of polyphenols primarily depends on the presence of multiple hydroxyl groups in their structure, which can form complexes with metal ions (Borowska et al., 2018). On the other hand, there are studies suggesting that, under the right conditions, polyphenols can promote mineral absorption processes (Sahraeian et al., 2024). Synergistic interactions between different dietary components may mitigate the potentially negative effects of polyphenols on mineral absorption. Furthermore, for ruminants, for which minerals are crucial for health and milk production, these effects may be of particular importance due to the role of the rumen and its microflora. Polyphenol degradation in the rumen is a complex process mostly depending on the chemical structure of polyphenols and the microbial environment within the ru-

men. Mostly tannins, but also some phenolic acids (such as ferulic acid and p-coumaric acid) are affecting their accessibility and degradation by rumen microbes. The degradation of these compounds is crucial as it impacts the digestibility of diet and the nutritional efficiency in ruminants. In our study, we used herbal mixtures with a high content of total polyphenols (average concentration was 2363.5 mg GAE/kg DM, with the highest content of 2786.7 mg in the D4 group), which contributed between 635 mg and 836 mg of total polyphenols per day per head. Compared to the herbal preparations used in the studies of other authors, the daily supply of polyphenols as active substances varies significantly from 18 mg to 550 mg/day/cow (Hashemzadeh-Cigari et al., 2014; di Meo et al., 2023). The results of our study did not show any negative effects of polyphenols on mineral availability; on the contrary, the groups supplemented with herbal mixtures showed an improvement in the mineral profile of the blood and milk of cows. It can result from the selection of ingredients in the blends, mainly low-tannin herbs.

Optimal nutrition with sufficient mineral levels is essential for the proper functioning of the organism, supporting structural, physiological, catalytic, and regulatory roles (Mordak et al., 2021). The differences in macronutrient concentrations in the cows' blood may be due to the level of their daily dietary intake, the amount of milk produced, and the physiological state of the animal (Nozad et al., 2012). Disorders resulting from deviations in macro- and micronutrient concentrations in the plasma of dairy cows are common, with the most common dysfunction being hypocalcaemia, i.e. low blood calcium levels (Kurek et al., 2015; Fu et al., 2022). Too low Ca supply, especially during early lactation, leads to Ca deficiency in the body and reduced milk production. The results of this study indicated a calcium deficit in the plasma of cows in the control group, compared to the experimental groups, which may impair the homeostatic mechanisms responsible for calcium balance (Gaignon et al., 2019; Mahen

et al., 2018). Besides calcium, crucial macronutrients to maintain health and high milk yields are phosphorus and magnesium. The analysis of the phosphorus content in the blood plasma of cows that were not supplemented with the herbal blends revealed their predisposition to a deficiency of the element. In comparison with the results of a study on Holstein-Friesian (HF) cows reported by Fadlalla et al. (2020), the present analyses showed lower concentrations of calcium and phosphorus in the blood plasma of the dairy cows from all the experimental groups in the early lactation period (Table 5). As reported by Winnicka (2021), the phosphorus content should range between 1.81 and 2.10 mmol/l. Disturbances in phosphorus metabolism in cows may also impair the digestibility of organic matter in the rumen and disturb absorption thereof in the gastrointestinal tract, which in turn may reduce the feed intake (Grünberg, 2023). Although, Keanthao et al. (2021) and Wächter et al. (2022) showed that the limitation of the phosphorus level in the feed ration for high-yielding cows in the transitional period had a beneficial effect on calcium homeostasis in the early stage of lactation. In the light of these results, diets lower in phosphorus than recommended may be possible, suggesting that a ration containing an average of 1.86 g P/kg dry matter may be sufficient for lactating dairy cows. In-house studies have shown the need for precise monitoring of levels of this element in feed to ensure healthy and high milk yielding cows (Table 4). Then, low phosphorus utilization efficiency of dairy cattle leads to excessive phosphorus excretion into the environment, negatively affecting water quality (Klop et al., 2014), an important environmental problem (Kebreab et al., 2008).

As shown in this study, the Mg^{2+} content in the blood serum of the experimental cows was within the normal physiological range specified by Winnicka (2021), however, significant differences were observed between the groups. It was in the range of 0.078–1.23 mmol/l, which reflects the current intake of this nutrient from the feed ration. As reported by Van Emon et al. (2020), sodium concentrations in the blood of dairy cows fluctuate depending on the animals' physiological status and age. The present study on HF cows in the early lactation stage has demonstrated that the supplementation with the herbal blend D4 contributes to the increase of the sodium level, compared to the control group. The optimal potassium level in the cow's body is a predictor of increased milk yield (Manuelian et al., 2018). In the present study, the potassium level in the blood plasma of all experimental animals was within reference values (Winnicka, 2021).

Analysis of mineral element content in the rations showed significant differences in the intake of microelements in experimental cows (Table 4). The husbandry system and the feeding regime have no effect on trace element content in plasma, as they are primarily a consequence of the low content of these elements in feed due to the low availability of trace elements in the soil (Dembek et al., 2014; Żarczyńska et al., 2017; Orjales et al., 2018). Optimal trace element concentrations have

a narrow range, and deviations from standard values can have a pronounced impact on overall animal health (Luna et al., 2019). Reproductive performance of cattle can be compromised if levels of zinc, copper or manganese, among others, are in the marginal to deficient range. Typical symptoms of copper deficiency in cattle include delayed or suppressed estrus, reduced fertility, infertility and embryo death. Also, inadequate zinc levels have been linked to reduced fertility, abnormal estrus, abortion and altered uterine myometrial contractility with prolonged parturition (Barros et al., 2018). The analyses performed in the present study showed that the concentrations of Cu, Fe, and Zn in the plasma of the experimental cows corresponded to the reference values (Winnicka, 2021), but the addition of herbs significantly affected their levels increasing their amount in blood plasma, particularly in the groups D2 and D4. There are no similar analyses of such relationships in the literature, but there are data on the correlations between elements in the blood serum (Fadlalla et al., 2020). The authors found a positive correlation between phosphorus and sodium and between magnesium and calcium as well as a negative correlation between calcium and potassium. Similarly, Pilarczyk et al. (2013) found a high Mg:Ca correlation and a positive Fe:Zn, Zn:K, and Ca correlations in the blood plasma of dairy cows.

Herbs by stimulation of animal health contribute to improving their production performance and the quality of animal-derived food products. In dairy cow nutrition, the focus is on their impact on milk quality parameters (Jarzynowska and Peter, 2017; Paskudzka et al., 2018), which corresponds to increasing consumer demands for raw material quality (Rabiei et al., 2021). Dietary supplementation of lactating cows with probiotics increases milk production and milk fat content (Hashemzadeh-Cigari et al., 2014). The positive impact of herbs on milk yield and composition inclusion may be related to the modification in rumen fermentation dynamics, increasing propionate synthesis and supporting lactation efficiency (Aschenbach et al., 2011). A similar effect in the basal composition of milk under the influence of the herbal mixture was observed in our previous study (Stobiecka et al., 2023). In the current study, we noticed an increase in milk yield among dairy cows that received supplementation with herbal mixtures. The milk yield noted especially in D2, D3, and D4 groups was increased, compared to the control group, but the changes were not significant ($P=0.066$). On the contrary, significant differences were observed in protein content as well as bacteria and somatic cell count (SCC). In all above-mentioned groups cows receiving herbal mixtures had increased protein content with a significant increase in D2, D3, and D4 groups. The best hygienic quality, with the lowest bacteria and somatic cell count, had the milk of cows receiving D2 mixture. The introduction of phenolic compounds that exhibit strong antioxidant properties may prove beneficial in protecting mammary gland cells from free radi-

cals, as the oxidative balance in the body may be compromised during mammary gland infection. In a study by Hashemzadeh-Cigari et al. (2014), supplementation with a phytobiotic supplement at 185 g per cow per day was shown to be an effective strategy for increasing milk yield and reducing SCC levels, particularly in cows with high levels of SCC in milk. These results are consistent with observations from our own study, in which higher milk production was also observed in cows from the experimental groups. At the same time, it was shown that cows in the control group and high SCC levels tended to have lower milk production compared to cows with significantly lower SCC levels.

The composition of micro- and macroelements contained in milk is often an integral part of their daily intake in the human diet. Therefore, their content in raw milk is increasingly being taken into account by cattle farmers with consideration of animal health and production performance as well as the quality of raw material and potential economic profits (Visentin et al., 2017; Denholm et al., 2019). An indicator of milk quality with high importance for the dairy industry is calcium content. As reported by Nogalska et al. (2017), the average calcium content in milk is 783.9 mg/l, whereas Mehra et al. (2021) estimated the level of this element at 1220 mg/l. The present study showed calcium levels in the range of 900 mg/l to 1090 mg/l, with a significantly higher amount of this element in the D1, D2, and D4 groups. Higher ration calcium supply in D4 cows has been shown to affect milk calcium content. Importantly, relatively low average calcium concentrations are observed in milk from Holstein-Friesian and mixed-breed cows (Dunsha et al., 2019). This may be a result of selection being carried out to increase milk volume in these breeds. In these studies, an increase in milk Ca content was observed as protein content increased. Other work confirms correlations between Ca and protein content in milk, as well as a significant relationship between calcium concentration and protein content, suggesting that higher protein content may favor greater calcium bioavailability (Davis et al., 2001; Bijl et al., 2013). Similar observations were made in our study; the results indicate that groups D1 and D4 achieved higher calcium concentrations in milk, which may be a consequence of their higher protein content. In the current study, sodium levels were similar in each experimental group and were lower as compared to the control group. This might be the reflection of higher milk yields observed in the experimental treatments in the study, as the content of sodium in cow's milk might be an important determinant of the intensity of milk synthesis. A study by Spek et al. (2012) showed an increase in dry matter intake (21.0–21.6 kg/d DM) and a decrease in ECM yield (27.1–25.1 kg/d) with increasing sodium (0.33% to 1.93% DM). Furthermore, Meir et al. (2023) recommend lowering the sodium content of the diet of cows at mid to late lactation in commercial herds to 0.52% dry matter to reduce the excretion of sodium into the environment with urine.

The average content of other minerals in milk found in this study agrees with other values reported in the literature (Stocco et al., 2019; NASEM, 2021; Saha et al., 2021), with exceptions regarding microelements like copper, iron, and zinc. Analysis of the concentrations of these minerals in milk and the values of Pearson's correlation coefficients, prove that herbs as a source of minerals might affect the concentration of microelements in milk. This observation is particularly important as the potential to enhance the concentration of micronutrients in milk is constrained by the intricate and well-coordinated biochemical processes of animal homeostasis (Sun et al., 2016). The presence of trace elements in milk has measurable health benefits for consumers, as they are enzymatic cofactors with an important role in human physiological processes. Copper is a vital element involved in many health-promoting processes, including antioxidant reactions (López-Alonso and Miranda, 2020). The present study has shown a higher concentration of this element in the milk from cows receiving herbal supplements, particularly in milk supplemented with D2, D3, and D4 mixtures. This finding is also confirmed by Proskura et al. (2017) and Pilarczyk et al. (2013). The zinc level in the milk was affected significantly by the inclusion of D3 and D4 herbal blends. Taking into account that zinc plays a crucial role in interacting with milk proteins, the inclusion of dairy products in the human diet may be a viable dietary strategy to improve zinc absorption (Shkembi and Huppertz, 2021; Shilpashree et al., 2022).

The study also showed a higher iron content in milk after the inclusion of herbs. A study by Kholif et al. (2021), conducted on lactating Friesian cows, showed that a phytobiotic supplement consisting of finely ground herbs and spices, administered at a dose of 3 g per cow per day, significantly increased ($P < 0.05$) the calcium (Ca) and zinc (Zn) content of milk. In addition, it was observed that a higher ration of the additive (6 g per cow per day) also contributed to a significant ($P < 0.05$) increase in the concentration of iron (Fe) and manganese (Mn) in milk. The concentration of iron observed in our study is higher than the results reported by Stocco et al. (2019) and Toscano et al. (2023), where the average iron content was 0.261 mg/kg and 0.280 mg/kg, respectively. But, in Dymtów and Włodarczyk (2022) study the average trace element concentrations in cow's milk were recorded as 0.03–0.06 mg/l for copper, 0.04–0.80 mg/l for iron, and 5.3–6.64 mg/l for zinc. In a separate study by Korać et al. (2023), it was observed that Cu and Zn levels in milk were 0.2–0.8 mg/kg and 10.75 mg/kg, respectively, indicating potential changes influenced by environmental conditions and cow diet. The results indicate that the results of our study fall in the given ranges, but analysis of the differences between the experimental groups shows a strong impact of individual blends and the possibility of modification of milk trace elements composition.

The observation of mineral concentrations within the normal range, especially in blood plasma, indicates sufficient mineral supplementation in their diet (Jacob et

al., 2002; Nozad et al., 2012). The values of the correlation coefficients and trends determined in the present study for the micro- and macroelements in the blood plasma and milk of the experimental cows confirm the multidirectional character and complexity of biological processes in which the nutrients are involved. They may result from similar physicochemical properties, multidirectional antagonistic or synergistic interactions, and coexistence and co-involvement with other micro- and macronutrients in physiological and metabolic processes, like competition for the site of absorption or enzymatic mechanisms (Khachlouf et al., 2019).

Conclusions

The study showed that herbs, besides being a source of bioactive compounds, should be considered a beneficial source of minerals, especially microelements like copper, zinc and iron, and might affect the mineral homeostasis in the organism. Considering that all cows received the same amounts of mineral and vitamin supplements in the basal feed, the observed changes must reflect the effect of the herbal mixtures. The use of herbs might optimize mineral uptake and metabolism, reducing the levels of undigested minerals excreted by cows and, consequently, minimizing nutrient runoff. Herbal blends affected not only the mineral profile of blood but most notably the milk mineral profile. Moreover, although the work focuses on the mineral profile, the changes were observed in other parameters, like milk's basic composition and its hygienic quality. This proves that minerals present in the diet might not only affect their content in the blood or milk, but they also play an important role as cofactors in many other biochemical processes involved in lactogenesis. The most significant results were achieved when blends of *Thymus vulgaris* L., *Echinacea purpurea* (L.) Moench, and *Origanum vulgare* L. were combined either with *Cinnamomum verum* J. Presl (D2 preparation) or with *Allium sativum* L., *Urtica dioica* L., *Salvia officinalis* L., *Matricaria chamomilla* L. (D4 preparation).

References

- Abo El-Maaty A.M., Aly M.A., Kotp M.S., Ali A.H., El Gabry M. A. (2021). The effect of Seasonal heat stress on oxidants-antioxidants biomarkers, trace minerals and acute-phase response of periparturient Holstein Friesian cows supplemented with adequate minerals and vitamins with and without retained fetal membranes. *Bull. Natl. Res. Cent.*, 45: 1–7.
- Aleri J.W., Hine B.C., Pyman M.F., Mansell P.D., Wales W.J., Mallard B., Fisher A.D. (2016). Periparturient immunosuppression and strategies to improve dairy cow health during the periparturient period. *Res. Vet. Sci.*, 108: 8–17.
- AlSuwaiegh S.B., Almotham A.M., Alyousef Y.M., Mansour A.T., Al-Sagheer A.A. (2022). Influence of functional feed supplements on the milk production efficiency, feed utilization, blood metabolites, and health of Holstein cows during mid-lactation. *Sustain Sci.*, 14: 8444.
- AOAC (2011). Association of Official Analytical Chemists. Official Methods of Analysis. Gaithersburg, MD, USA, 17th ed.
- Aschenbach J.R., Penner G.B., Stumpff F., Gäbel G. (2011). Ruminant nutrition symposium: role of fermentation acid absorption in the regulation of ruminal pH. *J. Anim. Sci.*, 89: 1092–1107.
- Ayrlle H., Schmid K., Disler M., Bischoff T., Stucki K., Zbinden M., Vogl C.R., Hamburger M., Walkenhorst M. (2015). Plant species reported from Swiss farmers to treat bovine respiratory diseases. *Planta Medica*, 81.
- Barros R.G., Lodde V., Dieci C., Fraciosi F., Luciano A.M. (2018). Study on the effects of zinc supplementation during *in vitro* embryo production technologies in cattle. *Proc. Veterinary and Animal Science Days 2018*, 6–8.06.2018, Milan, Italy.
- Bates A.J., Wells M., Laven R.A. (2022). The effect of pre-calving injection of trace mineral supplements on periparturient disease incidence in pasture based dairy cows. *Vet. J.*, 286: 105867.
- Bhatt N. (2015). Herbs and herbal supplements, a novel nutritional approach in animal nutrition. *Iran. J. Appl. Anim. Sci.*, 5: 497–516.
- Bijl E., van Valenberg H.J.F., Huppertz T., van Hooijdonk A.C.M. (2013). Protein, casein, and micellar salts in milk: Current content and historical perspectives. *J. Dairy Sci.*, 96: 5455–5464.
- Borowska S., Brzoska M.M., Tomczyk M. (2018). Complexation of bioelements and toxic metals by polyphenolic compounds – implications for health. *Curr. Drug Targets*, 19: 1612–1638.
- Cui Y., Shan Z., Hou L., Wang Q., Loo J.J., Xu C. (2021). Effect of natural Chinese herbal supplements (TCMF4) on lactation performance and serum biomarkers in periparturient dairy cows. *Front. Vet. Sci.*, 8: 801418.
- Daniel J.B., Kvidera S.K., Martín-Tereso J. (2020). Total-tract digestibility and milk productivity of dairy cows as affected by trace mineral sources. *Int. J. Dairy Sci.*, 103: 9081–9089.
- Davis S.R., Farr V.C., Knowles S.O., Lee J., Kolver E., Auldust M.J. (2001). Sources of variation in milk calcium content. *Aust. J. Dairy Tech.*, 56: 156.
- Del Río-Avilés A.D., Correa-Calderón A., Avendaño-Reyes L., Macías-Cruz U., Thomas M.G., Enns R.M., Speidel S.E., Sánchez-Castro M.A., Zamorano-Algandar R., López-Castro P.A., Luna-Nevárez P. (2022). Mineral supplementation (injectable) improved reproductive performance in Holstein cows managed in a warm summer environment. *Reprod. Domest. Anim.*, 57: 839–848.
- Dembek R., Łyszczarz R., Zimmer-Grajewska M. (2014). *Plantago lanceolata* L. as a component of permanent and renewed grasslands. *Acta Sci. Pol. Agric.*, 13: 19–30.
- Denholm S.J., Sneddon A.A., McNeilly T.N., Bashir S., Mitchell M.C., Wall E. (2019). Phenotypic and genetic analysis of milk and serum element concentrations in dairy cows. *J. Dairy Sci.*, 102: 11180–11192.
- Di Meo M.C., Salzano A., Zotti T., Palladino A., Giaquinto D., Maruccio L., Varricchio E. (2023). Plasma fatty acid profile in Italian Holstein-Friesian dairy cows supplemented with natural polyphenols from the olive plant *Olea Europaea* L. *Vet. and Anim. Sci.*, 21: 100298.
- Dunshea F.R., Walker G.P., Williams R., Doyle P.T. (2019). Mineral and citrate concentrations in milk are affected by seasons, stage of lactation and management practices. *Agriculture*, 9: 25.
- Dymtów I., Włodarczyk K. (2022). Composition and nutritional value of mare and donkey milk compared to milk from cows. *Food. Sci. Technol. Qual.*, 27: 28–39.
- El-Saadony M.T., Yang T., Saad A.M., Alkafaas S.S., Elkafas S.S., Eldeeb G.S., Lorenzo J.M. (2024). Chemistry, bioavailability, bioactivity, nutritional aspects and human health benefits of polyphenols: A comprehensive review. *Int. J. Biol. Macromol.*, 134223.
- Erickson P.S., Kalscheur K.F. (2020). Nutrition and feeding of dairy cattle. *Anim. Agric.*, 157–180.
- Ezzat Abd El-Hack M., Alagawany M., Ragab Farag M., Tiwari R., Karthik K., Dhama K., Adel M. (2016). Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. *J. Essent. Oil Res.*, 28: 365–382.
- Fabjanowska J., Kowalczyk-Vasilev E., Klebaniuk R., Milewski S., Gümüş H. (2023). N-3 polyunsaturated fatty acids as a nutritional support of the reproductive and immune system of cattle – a review. *Animals*, 13: 3589.

- Fabjanowska J., Kowalczyk-Vasilev E., Klebaniuk R. (2024). Effectiveness of the use of herbal mixtures in feeding calves of meat breeds (in Polish). *Sust. Anim. Prod.*, 49.
- Fadlalla I.M.T., Omer S.A., Atta M. (2020). Determination of some serum macroelement minerals levels at different lactation stages of dairy cows and their correlations. *African J. Sci.*, 8: e00351.
- Fu Y., Colazo M.G., De Buck J. (2022). Development of a blood calcium test for hypocalcemia diagnosis in dairy cows. *Res. Vet. Sci.*, 147: 60–67.
- Gaignon P., Le Grand K., Laza-Knoerr A.L., Hurtaud C., Boudon A. (2019). Effect of calcium intake and the dietary cation-anion difference during early lactation on the bone mobilization dynamics throughout lactation in dairy cows. *PLoS One*, 14(11): e0218979.
- Goff J.P. (2018). Invited review: Mineral absorption mechanisms, mineral interactions that affect acid-base and antioxidant status, and diet considerations to improve mineral status. *J. Dairy Sci.*, 101: 2763–2813.
- Grünberg W. (2023). Phosphorus metabolism during transition. *Vet. Clin. N. Am. – Food Anim. Pract.*, 39: 261–274.
- Harvey K.M., Cooke R.F., Marques R.D.S. (2021). Supplementing trace minerals to beef cows during gestation to enhance productive and health responses of the offspring. *Animals*, 11: 1159.
- Hashemzadeh-Cigari F., Khorvash M., Ghorbani G.R., Kadivar M., Riasi A., Zebeli Q. (2014). Effects of supplementation with a phytobiotics-rich herbal mixture on performance, udder health, and metabolic status of Holstein cows with various levels of milk somatic cell counts. *J. Dairy Sci.*, 97: 7487–7497.
- INRA (2019). INRA feeding system for ruminants (2nd ed). Wageningen Academic Publishers, Wageningen, the Netherlands, 640 pp.
- Jacob M.P., Cazaubon M., Scemama A., Prié D., Blanchet F., Guillin M.C., Michel J.B. (2002). Plasma matrix metalloproteinase-9 as a marker of blood stasis in varicose veins. *Circulation*, 106: 535–538.
- Jarzynowska A., Peter E. (2017). Effect of adding herbs to winter diet on fatty acid profile of lipid fraction of sheep milk (in Polish). *Rocz. Nauk. Pol. Tow. Zootech.*, 13: 43–54.
- Keanthao P., Goselink R.M.A., Dijkstra J., Bannink A., Schonewille J.T. (2021). Effects of dietary phosphorus concentration during the transition period on plasma calcium concentrations, feed intake, and milk production in dairy cows. *J. Dairy Sci.*, 104: 11646–11659.
- Kebreab E., Odongo N.E., McBride B.W., Hanigan M.D., France J. (2008). Phosphorus utilization and environmental and economic implications of reducing phosphorus pollution from Ontario dairy cows. *J. Dairy Sci.*, 91: 241–246.
- Khachlouf K., Hamed H., Gdoura R., Gargouri A. (2019). Effects of dietary zeolite supplementation on milk yield and composition and blood minerals status in lactating dairy cows. *J. Appl. Anim.*, 47: 54–62.
- Kholif A.E., Hassan A.A., El Ashry G.M., Bakr M.H., El-Zaiat H.M., Olafadehan O.A., Sallam S.M.A. (2021). Phyto-genic feed additives mixture enhances the lactational performance, feed utilization and ruminal fermentation of Friesian cows. *Anim. Biotech.*, 32: 708–718.
- Kiczorowska B., Samolińska W., Al-Yasiry A.R.M., Kiczorowski P., Winiarska-Mieczan A. (2017). The natural feed additives as immunostimulants in monogastric animal nutrition—a review. *Ann. Anim. Sci.*, 17: 605–625.
- Kikusato M. (2021). Phytobiotics to improve health and production of broiler chickens: functions beyond the antioxidant activity. *Anim. Biosci.*, 34: 345.
- Klebaniuk R., Grela E.R., Kowalczyk-Vasilev E., Olcha M., Gózdź J. (2014). Effectiveness of using herbal mixtures in organic cattle breeding (in Polish). *Wiad. Zoot.*, 52: 56–63.
- Klebaniuk R., Kowalczyk-Vasilev E., Bąkowski M., Rocki G., Grela E.R., Kiczorowska B., Matras J., Widz J., Kepka K. (2017). Effectiveness of herbal mixture use (in Polish). *Med. Weter.*, 73: 751–755.
- Klop G., Ellis J.L., Blok M.C., Brandsma G.G., Bannink A., Dijkstra J. (2014). Variation in phosphorus content of milk from dairy cattle as affected by differences in milk composition. *J. Agric. Sci.*, 152: 860–869.
- Korać R.S., Huremović J., Žero S., Ljubijankić N. (2023). Content of metals in cow, sheep and goat milk samples. *Bull. Chem. Technol. Bosnia Herzeg.*, 60: 1–6.
- Kozłowska M., Laudy A.E., Przybył J., Ziarno M., Majewska E. (2015). Chemical composition and antibacterial activity of some medicinal plants from the Lamiaceae family. *Acta Pol. Pharm.*, 72: 757–767.
- Krawęcka A., Sobota A., Ivanišová E., Harangozo L., Valková V., Zielińska E., Mildner-Szkudlarz S. (2022). Effect of black cumin cake addition on the chemical composition, glycemic index, antioxidant activity, and cooking quality of durum wheat pasta. *Molecules*, 27: 6342.
- Kumar M., Kumar V., Roy D., Kushwaha R., Vaiswani S. (2014). Application of herbal feed additives in animal nutrition – a review. *Int. J. Livest. Res.*, 4: 1–8.
- Kuralkar P., Kuralkar S.V. (2021). Role of herbal products in animal production – an updated review. *J. Ethnopharmacol.*, 278: 114246.
- Kurek Ł., Lutnicki K., Kluciński W., Kleczkowski M., Brodzki P., Marczuk J., Gołyński M. (2015). Effect of hypocalcemia on parenchymal organ function in cows at the peak of lactation (in Polish). *Med. Weter.*, 71: 307–311.
- López-Alonso M., Miranda M. (2020). Copper supplementation, a challenge in cattle. *Animals*, 10: 1890.
- Luna D., López-Alonso M., Cedeño Y., Rigueira L., Pereira V., Miranda M. (2019). Determination of essential and toxic elements in cattle blood: serum vs plasma. *Animals*, 9: 465.
- Mahen P.J., Williams H.J., Smith R.F., Grove-White D. (2018). Effect of blood ionized calcium concentration at calving on fertility outcomes in dairy cattle. *Vet. Rec.*, 83: 263.
- Manuelian C.L., Penasa M., Visentin G., Zidi A., Cassandro M., De Marchi M. (2018). Mineral composition of cow milk from multi-breed herds. *Anim. Sci. J.*, 89: 1622–1627.
- Mascitelli L., Goldstein M.R. (2014). Inhibition of iron absorption by polyphenols as an anticancer mechanism. In: *Polyphenols in Human Health and Disease*. Acad. Press, pp. 1283–1286.
- Mehra R., Singh R., Nayan V., Buttar H.S., Kumar N., Kumar S., Kumar H. (2021). Nutritional attributes of bovine colostrum components in human health and disease: a comprehensive review. *Food Biosci.*, 40: 100907.
- Meir Y.A.B., Shaani Y., Bikel D., Portnik Y., Jacoby S., Moallem U., Frank E. (2023). Reducing dietary sodium of dairy cows fed a low-roughages diet affect intake and feed efficiency, but not yield. *Anim. Nutr.*, 12: 1–6.
- Mirowski A. (2019). Utility of herbs in cattle nutrition (in Polish). *Żywiec Weter.*, 92: 131–133.
- Mordak R., Dobrzański Z., Kupczyński R. (2021). Relationships among macro-minerals, other selected serum markers of bone profile and milk components of dairy cows during late lactation. *Ann. Anim. Sci.*, 21: 887–898.
- Mutepawar S.S., Jadhav S.B., Kankudate A.D., Sanghai S.D., Usturge D.R., Chavare S.S. (2014). A review on bioavailability enhancers of herbal origin. *Word J. Pharm. Sci.*, 3: 667–677.
- NASEM (2021). National Academies of Sciences, Engineering, and Medicine. *Nutrient Requirements of Dairy Cattle: Eighth Revised Edition*. Washington, DC: The National Academies Press.
- Nogalska A., Momot M., Sobczuk-Szul M., Pogorzelska-Przybyłek P., Nogalski Z. (2017). Calcium and magnesium content in the milk of high-yielding cows. *J. Elem.*, 22: 809–815.
- Nozad S., Ramin A.G., Moghadam G., Asri-Rezaei S., Babapour A., Ramin S. (2012). Relationship between blood urea, protein, creatinine, triglycerides and macro-mineral concentrations with the quality and quantity of milk in dairy Holstein cows. *Vet. Res. Forum*, 3: 55.
- Orjales I., Herrero-Latorre C., Miranda M., Rey-Crespo F., Rodríguez-Bermúdez R., López-Alonso M. (2018). Evaluation of trace element status of organic dairy cattle. *Animal*, 12: 1296–1305.
- Paskudzka A., Kołodziejczyk D., Socha S. (2018). The use of herbs in animals. *Acta Sci. Pol. Zootech.*, 17: 3–14.
- Pilarczyk J., Wójcik P., Czerniak P., Sablik B., Pilarczyk A., Tomza-Marciniak A. (2013). Concentrations of toxic heavy metals and trace elements in raw milk of Simmental and Holstein-Friesian cows from organic farm. *Environ. Monit. Assess.*, 185: 8383–8392.

- Proskura N., Podlasińska J., Proskura W.S., Frost-Rutkowska A., Dybus A., Szydłowski K. (2017). Concentrations of macroelements and trace elements in milk of Jersey cows. *Indian J. Anim. Res.*, 51: 89–92.
- Puppel K., Kuczyńska B. (2016). Metabolic profiles of cow's blood; a review. *J. Sci. Food Agric.*, 96: 4321–4328.
- Rabiei S., Zahedi M., Abtahi M., Doustmohammadian A., Dadkhah M., Zoghi T., Hajigholam-Saryazdi M. (2021). Consumption of milk and dairy products in Iranian population; barriers and facilitators. *Clin. Nutr. Exp.*, 38: 1–23.
- Redoy M.R.A., Shuvo A.A.S., Cheng L., Al-Mamun M. (2020). Effect of herbal supplementation on growth, immunity, rumen histology, serum antioxidants and meat quality of sheep. *Animal*, 14: 2433–2441.
- Rivera-Chacon R., Castillo-Lopez E., Ricci S., Petri R.M., Reisinger N., Zebeli Q. (2022). Supplementing a phytogenic feed additive modulates the risk of subacute rumen acidosis, rumen fermentation and systemic inflammation in cattle fed acidogenic diets. *Animals*, 12: 1201.
- Saha S., Piazza M., Bittante G., Gallo L. (2021). Macro- and micro-mineral composition of milk from purebred Holsteins and four generations of three-breed rotational crossbred cows from Viking Red, Montbéliarde and Holstein sires. *Ital. J. Anim. Sci.*, 20: 447–452.
- Sahraeian S., Rashidinejad A., Golmakani M.T. (2024). Recent advances in the conjugation approaches for enhancing the bioavailability of polyphenols. *F. Hydro.*, 146: 109221.
- Seyfaddinov S. (2022). What is the share of balanced quality feed? *J. Appl. Sci.*, 5: 21–26.
- Shan C.H., Guo J., Sun X., Li N., Yang X., Gao Y., Zhao J.J. (2018). Effects of fermented Chinese herbal medicines on milk performance and immune function in late-lactation cows under heat stress conditions. *J. Anim. Sci.*, 96: 4444–4457.
- Sharma N., Kundu S.S., Tariq H., Mani V., Malhotra R. (2021). Effect of fat and protein along with polyherbal preparation on reproductive health of periparturient Karan Fries cows. *Indian J. Anim. Res.*, 55: 657–62.
- Shilpashree B.G., Arora S., Chawla P., Sharma V. (2022). A comparison of zinc interactions with succinylated milk protein concentrate and sodium caseinate. *LWT*, 157: 113116.
- Shkembi B., Huppertz T. (2021). Influence of dairy products on bioavailability of zinc from other food products: A review of complementarity at a meal level. *Nutrition*, 13: 4253.
- Singleton V.L., Rossi J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 16: 144–158.
- Spek J.W., Bannink A., Gort G., Hendriks W.H., Dijkstra J. (2012). Effect of sodium chloride intake on urine volume, urinary urea excretion, and milk urea concentration in lactating dairy cattle. *J. Dairy Sci.*, 95: 7288–7298.
- Stobiecka M., Król J., Brodziak A., Klebaniuk R., Kowalczyk-Vasilev E. (2023). Effects of supplementation with an herbal mixture on the antioxidant capacity of milk. *Animals*, 13: 2013.
- Stocco G., Summer A., Malacarne M., Cecchinato A., Bittante G. (2019). Detailed macro-and micromineral profile of milk: Effects of herd productivity, parity, and stage of lactation of cows of 6 dairy and dual-purpose breeds. *J. Dairy Sci.*, 102: 9727–9739.
- Strzetelski J.A., Brzóska F., Kowalski Z.M., Osieglowski S. (2014). Nutritional recommendations for ruminants and feed value tables (in Polish). Kraków, Poland, National Research Institute of Animal Production, 287 pp.
- Sun F., Cao Y., Cai C., Li S., Yu C., Yao J. (2016). Regulation of nutritional metabolism in transition dairy cows: Energy homeostasis and health in response to post-ruminal choline and methionine. *PLoS one*, 11(8), e0160659.
- Tamminen L.M., Emanuelson U., Blanco-Penedo I. (2018). Systematic review of phytotherapeutic treatments for different farm animals under European conditions. *Front. Vet. Sci.*, 5: 140.
- Toscano A., Giannuzzi D., Pegolo S., Vanzin A., Bisutti V., Gallo L., Trevisi E., Cecchinato A., Schiavon S. (2023). Associations between the detailed milk mineral profile, milk composition, and metabolic status in Holstein cows. *J. Dairy Sci.*, 106: 6577–6591.
- Tsiamadis V., Banos G., Panousis N., Kritsepi-Konstantinou M., Arsenos G., Valergakis G.E. (2016). Genetic parameters of calcium, phosphorus, magnesium, and potassium serum concentrations during the first 8 days after calving in Holstein cows. *J. Dairy Sci.*, 99: 5535–5544.
- Tuhy Ł., Dmytryk A., Samoraj M., Chojnacka K. (2018). Trace elements in animal nutrition. In: Recent advances in trace elements, Chojnacka K., Saeid A. (eds). Wiley Blackwell, pp. 319–337.
- Van Emon M., Sanford C., McCoski S. (2020). Impacts of bovine trace mineral supplementation on maternal and offspring production and health. *Animals*, 10: 2404.
- Visentin G., De Marchi M., Berry D.P., McDermott A., Fenelon M.A., Penasa M., McParland S. (2017). Factors associated with milk processing characteristics predicted by mid-infrared spectroscopy in a large database of dairy cows. *J. Dairy Sci.*, 100: 3293–3304.
- Wächter S., Cohrs I., Golbeck L., Wilkens M.R., Grünberg W. (2022). Effects of restricted dietary phosphorus supply to dry cows on periparturient calcium status. *J. Dairy Sci.*, 105: 748–760.
- Wilkens M.R., Muscher-Banse A.S. (2020). Regulation of gastrointestinal and renal transport of calcium and phosphorus in ruminants. *Animal*, 14: 29–43.
- Winnicka A. (2021) Reference values for basic laboratory tests in veterinary medicine (in Polish). SGGW, Warsaw, Poland, 113 pp.
- Wójtowski J.A., Majcher M., Danków R., Pikul J., Mikołajczak P., Molińska-Glura M., Stanisławski D. (2023). Effect of herbal feed additives on goat milk volatile flavor compounds. *Foods*, 12: 2963.
- Xie Y., Chen Z., Wang D., Chen G., Sun X., He Q., Sun J. (2020). Effects of fermented herbal tea residues on the intestinal microbiota characteristics of Holstein heifers under heat stress. *Front. Microbiol.*, 11: 1014.
- Yaremchuk O.S., Farionik T.V. (2022). Effect of chelate compounds of microelements on the organism of agricultural animals. *Mod. Eng. Innov. Technol.*, 23: 99–115.
- Yazlık M.O., Çolakoğlu H.E., Pekcan M., Kaya U., Küplülü Ş., Kaçar C., Vural M.R. (2021). Effects of injectable trace element and vitamin supplementation during the gestational, peri-parturient, or early lactational periods on neutrophil functions and pregnancy rate in dairy cows. *Anim. Reprod. Sci.*, 225: 106686.
- Żarczyńska K., Żarczyński P., Sobiech P., Snarska A., Stopyra A., Wieteska M., Płaczek A. (2017). The effect of micronutrient deficiencies on the health status of transition cows. *J. Elem.*, 22: 1223–1234.

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