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Multivariate statistical assessment of regional differences in mineral composition of cow's milk and feed in Kosovo

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Abstract

This study investigated regional differences in the composition of raw cow's milk and feed to determine their interrelationship using multivariate analysis. The study included 104 farms producing raw cow's milk (with more than 10 dairy cows) across seven regions of Kosovo during the 2022-2024 period. Physicochemical parameters of milk (pH, density, acidity, fat, lactose, protein, water activity (aw), solids-not-fat (SNF), conductivity, FP and ash) were analyzed, while macroelements (Ca, K, P, Mg, Na) and microelements (Fe, Zn, Cu, Mn) were determined in both milk and feed. Statistical analysis included descriptive statistics and one-way ANOVA (with Tukey's HSD post-hoc test) to determine regional differences. Principal component analysis (PCA) was applied to investigate multivariate effects and relationships between milk and feed parameters. Statistically significant regional differences were confirmed in most of the observed physicochemical and mineral parameters of raw cow's milk. The average mineral composition of milk from Kosovo dairy farms showed that higher concentrations of phosphorus (P) and sodium (Na) were observed relative to the internationally recommended values (FAO), while the concentrations of Ca, K, Mg, Zn and Mn were lower than recommended. A positive correlation was observed between Mg and Ca in animal feed and milk density, which was confirmed by Pearson correlation coefficients r_{Ca}= 0.787 and r_{Mg}=0.767. The application of multivariate analysis (MVA) was extremely important to obtain an integrated assessment of nutrient transfer and regional differentiation. Overall, the findings highlight that improving feed formulation and mineral balance could improve the nutritional quality of milk, provide a scientific basis for regional dairy product branding initiatives and promote sustainable livestock management.

Keywords: cow's milk; animal feed; milk quality; multivariate analysis; mineral composition

Introduction

Milk is the first food consumed by most people around the world from the moment of birth, thus becoming a vital chain of survival for many of them (Castellini and Graffigna, 2022). The consumption of milk and dairy products is mainly based on their high nutritional and caloric value such as proteins and fats but also mineral composition such as calcium, potassium, and vitamin D (Gantner et al., 2024; Bórawski et al., 2020). At the European Union level, the largest milk producers are Germany, France, Poland, the Netherlands, and Italy, accounting for approximately 70 % of the total production. Milk production in the EU has increased over recent decades, rising from 151 million tons in 1998 to over 165 million tons in 2017, an increase of about 10 % (Mihai et al., 2023). Milk production in Kosovo has remained relatively stable, reaching 276,058 tons in 2023 (MBPZHR, 2023). Trends in milk production growth are attributed to several factors, such as: breed, genetics, heat stress (HS), environmental conditions, and mixed feed for dairy cows (Chavarría et al., 2025). Studies indicate that a negative energy balance in rations affects both milk yield and animal health (Leduc et al, 2021; Ivanković et al., 2021; Gantner et al, 2024a; Mićić et al., 2022). Research indicates that the use of total mixed ration (TMR) is a key factor influencing milk production in dairy cows (Pastorini et al, 2019; Gulati et al., 2018). The combination of diet and breed is known to significantly influence the physicochemical properties and mineral composition of raw milk. Studies have shown that different mixed feeding systems for animals can also determine the increase in macromineral values in raw milk (Gulati et al., 2018; Saha et al., 2021; Manuelian et al., 2018; Teter et al., 2020). Also, in a study in the United Kingdom was found that mixed feeds with a composition of forages and other additives contribute more to increasing the mineral values in animal feed, including P, and Ca (Sinclair and Atkins, 2015). Another study emphasizes the increase in milk yield and quality through precise feed formulation using advanced techniques (e.g., machine learning, optimization algorithms, multivariate analysis, etc.), and highlights that such an approach improves the productivity and sustainability of the milk production process (Akintan et al., 2025). Considering the current state of knowledge, research on the relationship between milk composition and animal nutrition constitutes an important contribution to understanding milk quality. Therefore, this study aimed to assess raw milk production across the entire territory of the Republic of Kosovo, using a large number of samples collected from various farms. The research encompassed all seven regions of Kosovo and included comparisons with results reported in studies conducted both regionally and internationally.

Materials and methods

Research area and sample collection

The study included 104 farms producing raw cow's milk across the territory of Kosovo, with a focus on large farms (i.e. with more than 10 dairy cows). The farms were distributed across seven regions of Kosovo, as presented in table 1, which also summarizes the number of farms and the distribution of dairy cattle breeds by region. The lowest number of cows was recorded in region 5 (n=270) while the highest was recorded in region 3 (n=800).

Milk sampling

Raw cow's milk samples were collected directly from 104 farms. Three samples were obtained from each farm, resulting in a total of 312 samples. Samples were collected in sterile 500 mL containers and transported to the laboratory in refrigerated boxes maintained at 5–8 °C. The sampling and transport procedures were conducted in accordance with international standards (ISO 707:2008; ISO/TC 34/SC 5, 2016; Tamime 2009).

Animal feed sampling

Animal feed samples were collected from the same 104 farms, yielding a total of 312 samples (3 samples per farm, each weighing approximately 1 kg). Samples were taken from three different points of the feed mixer, thoroughly homogenized, and dried under laboratory conditions prior to analysis (Malomo and Ihegwuagu, 2017).

Analytical methods

The following physicochemical and mineral parameters were analyzed:

- Milk physicochemical parameters: pH; density (g/cm^3); acidity (°SH); fat (%); lactose (%); protein (%); solids-not-fat (SNF, %); water activity (Aw); freezing point (°C);

Table 1. Overview of farms included in the study, including herd size ranges and dairy cattle breeds per farm

Animal/Breed (%)	**Region 1** (Prishtina)	**Region 2** (Mitrovica)	**Region 3** (Peja)	**Region 4** (Prizren)	**Region 5** (Ferizaj)	**Region 6** (Gjilan)	**Region 7** (Gjakova)
Holstein	41	26	45	51	32	43	50
Simmental	56	74	56	39	59	56	50
Montbéliarde	3	0	0	3	0	1	0
Busha	0	0	0	0	9	0	0
Montafon	0	0	0	7	0	0	0
Number of farms (cows per farm)	14 (15-70)	14 (10-70)	20 (10-100)	15 (10-70)	11 (10-60)	16 (10-140)	14 (14-70)

conductivity (mS/cm); and ash (%)

- Mineral composition (milk and animal feed): macrominerals (Ca, K, P, Mg, Na) and microminerals (Fe, Zn, Cu, Mn)
- Animal feed physicochemical parameters: protein (%), fat (%), and ash (%).

All analyses were performed at the laboratories of the Agricultural Institute of Kosovo and the Faculty of Agribusiness, University of Peja, following standardized methods.

Milk analysis

The pH of milk samples was measured using a HANNA- HI 5221 (USA, EU- Romania) and an Isolab stirrer (Laborgerate GmbH-LCD, Germany). The instruments were calibrated with standard solutions before each sampling with an accuracy of ± 0.01 pH at 20 °C (Yerlikaya et al., 2020; Anika et al., 2023). Milk density was determined using a portable Density2Go (Mettler Toledo, Switzerland) with an accuracy of ±0.001 g/cm^3. Samples were analyzed at 20 °C, and results were obtained within 30 seconds (Parmar et al., 2021). Titratable acidity (TA) was determined using the Soxhlet Henkel method (AOAC Official Method 947.05), by titrating the samples with 0.1 M NaOH in the presence of 1 mL of (2 % w/v) phenolphthalein solution (Yerlikaya et al., 2020). Milk fat content was determined using the Gerber method (AOAC 2000.18; ISO 19662:2018). Protein content was determined using the Kjeldahl method (AOAC 991.20) applying a conversion factor (eq. 2) of 6.38 for milk and dairy products (Bendelja Ljoljić et al., 2023; Ceren Akal, 2023). Lactose content, freezing point and Solids-Non-Fat (SNF) were determined using Laktoscan SA and Ekomilk- ultrasonic milk analyzers (Ltd Bulgaria) according to Kaya et al.2023). Water activity (aw) was measured using a Hygropal Retronic -HP 23-AW (UK, England) in accordance with ISO 18787:2017 (Baranowska et al., 2017). Electrical Conductivity (EC) was measured at 25 °C using Mettler Toledo FEP30 Instrument (USA) calibrated according to Anika et al. (2023). Ash content was determined using the gravimetric method (AOAC 945.46; Rațu et al., 2021), involving drying at 103 °C - 105 °C ± 1 °C, followed by incineration at 550±20 °C for about 3 hours using a muffle furnace (ISO-1105-S-B2, IsoLab Laborgerate GmbH, Turkey) oven was used. The obtained values were substituted into the following formula (equation 1):

$$Ash\ (\%) = (W_3 - W_1) \cdot 100 / (W_2 - W) \qquad (1)$$

where: W_1 = mass of empty dish (g), W_2 = mass of the dish and sample (g), and W_3 = mass of dish and residue after incineration (g).

Animal feed analysis

The determination of protein in animal feed was adapted according to the standards (AOAC 988.05; AOAC 984.13 and Ahmed et al.,2021; FAO, 2011): 0.5-1 g of animal feed ground to a particle size of approximately 2 mm was digested in the Infrared Digestion Unit (Beger IDU, Slovenia) in the presence of catalysts (VW A00000282, VWR- Leuven Belgium), H_2SO_4 (95-98 % v/v, Sigma-Aldrich-Germany) and H_2O_2 (30-35 % v/v, Lach-Ner, Czech Republic). The digested samples were distilled using a Velp UDK 149 Kjeldahl apparatus (Italy) in the presence of solvents. The obtained values were substituted into the formula (equation 2), using a factor of F = 6.25 for all forages, feeds, and mixed feeds.

$$Protein\ (\%\ CP) = \%\ N \cdot F \qquad (2)$$

The determination of fat in animal feed was performed using Soxtherm-Gerhardt-414 automatic extraction system (Germany) with some modifications (AOAC 922.06; AOAC 920.85; Nacu et al., 2021; FAO, 2011). 2-3 grams of feed in the presence of 50-70 mL of Diethyl Ether solvent (Lach-Ner, Czech Republic) were placed in the automatic extraction system (Soxtherm-Gerhardt-414), program: (classification T=135 °C; extraction temperature= 125 °C; reduction interval= 3min and 30s; reduction pulse= 1 s; hot extraction=0 h and 30 min; evaporation A= interval 5x; extraction time= 1 h and 20 min; evaporation B= interval 3.0x; evaporation C= 10 min; program duration= 2 h and 28 min). The obtained values were substituted into the formula (equation 3):

$$Fat\ (\%) = (A_1 - A_0) \cdot 100 / m \qquad (3)$$

where: A_0=extraction beaker (g); A_1= extraction beaker with fat (g); m= sample weight (g)

Determination of ash in animal feed was performed using the gravimetric method (AOAC 942.05), following the procedure described by Thiex et al. (2012) and FAO (2011).

Sample preparation for mineral analysis

Raw milk sample preparation

1 mL of raw milk was transferred into a Teflon (PTFE) digestion tube. Then, 6 mL of HNO_3 (69 % v/v, ultrapure, Sigma-Aldrich, Germany), 1 mL of H_2O_2 (30-35 % v/v, Lach-Ner, Czech Republic), and 1 mL of HCl (32-36 % v/v, Sigma-Aldrich, Germany) were added. The mixture was digested using a microwave digestion system (ETHOS UP, Italy). After digestion, the samples were diluted in plastic containers to a final volume of 50 mL using deionized water. Mineral analysis was performed using Microwave Plasma-Atomic Emission Spectroscopy (MP-AES, Agilent 4200 MP-AES, USA) (Teklu et al., 2023; Pastorelli et al., 2023). A multi-element IV standard solution for ICP (Merck, Darmstadt, Germany) was used for instrument calibration.

Animal feed sample preparation

A portion of 0.18-0.5 g of dry, finely ground animal feed was placed into a Teflon (PTFE) digestion tube. Then, 7 mL of HNO_3 (69 % v/v, Sigma-Aldrich, Germany) and 1 mL of H_2O_2 (30-35 % v/v, Lach-Ner, Czech Republic) were added. The mixture was digested using a microwave digestion system (ETHOS UP, Italy). After digestion, the samples were diluted with ultrapure water in plastic containers to a final volume of 50 mL. Mineral analysis was performed following the procedure described for milk samples (Liberato et al., 2017).

Statistical analysis

The obtained data were analyzed using both descriptive and inferential statistical methods. Descriptive statistics included mean values, standard deviations (SD), and the minimum and maximum values. Prior to analysis, continuous variables were screened for outliers and tested for normality using the Shapiro-Wilk test. One-way ANOVA followed by Tukey's HSD post-hoc test was used to determine statistically significant differences between regions (with a significance level of $p<0.05$). Results are presented as mean ± SD. Means sharing the same superscript letter are not significantly different.

Multivariate statistical analysis

To investigate the multivariate relationships, multivariate tools were used. Principal component analysis (PCA) was applied to milk and feed data to identify underlying patterns and associations. Dendrograms and heatmaps were used to visualize clustering of regions and variables based on compositional similarities (for milk and animal feed). The statistical analyses and graphical visualizations were performed using Python and SPSS software.

Results and discussion

The results are divided into three sections: (i) milk analysis, (ii) animal feed analysis and (iii) relationship between milk parameters and feed quality.

Milk analysis

The results of the physicochemical parameters of cow's milk, expressed as mean ± standard deviation (SD), for 11 parameters collected from farms across seven regions of Kosovo are presented in Table 2.

The observed regional differences in milk composition, particularly in fat, protein, and solids-not-fat (SNF) content, can be partially associated with differences in feeding practices and farm management across Kosovo. However, these relationships should be interpreted with caution, as milk composition is influenced by a complex interaction of nutritional, physiological, and environmental factors. In this context, it is important to emphasize that the mineral composition of milk is regulated by homeostatic mechanisms within the mammary gland. These mechanisms maintain relatively stable concentrations of key ions such as Ca, P, Na, K, and Mg across a wide range of dietary conditions (Gaucheron, 2005; Shennan and Peaker, 2000; Fox and McSweeney, 2015). Consequently, under normal and balanced feeding conditions, variations in mineral intake through feed generally result in only minor or biologically limited changes in the mineral composition of milk. This indicates that the mineral profile of milk is not a direct reflection of daily dietary mineral variability, but rather a result of tightly controlled physiological processes associated with lactation. While similar patterns of regional variability have been reported in other studies, including those conducted in Peru (Bardales et al., 2024), the present study extends these findings by combining milk and feed data within a multivariate framework. This approach allows for a more comprehensive understanding of the relationships between diet composition and milk quality, moving beyond simple descriptive comparisons toward a mechanistic interpretation. The present study demonstrated a similar pattern.

Figures 1 and 2 illustrate the distribution of the physicochemical parameters of cow's milk analyzed in this study, complementing the mean values and standard deviations (SD) for Kosovo. Boxplots, histograms, and violin plots were used to visualize the distributions. The regional differences in the mineral content of cow's milk are presented in table 3. Figure 1 specifically shows the distribution of the main physicochemical parameters, including pH, density, acidity, fat content, lactose content, and protein content.

Nine minerals were analyzed in the milk samples, and their mean values are presented in Table 3.

Table 2. Physicochemical parameters of cow's milk in different regions of Kosovo

Parameters	Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7
pH	6.83±0.22^{a}	6.95±0.17^{a}	6.92±0.18^{a}	6.96±0.11^{a,b}	6.94±0.12^{a}	6.97±0.07^{b}	6.94±0.15^{a}
Density (g/cm^{3})	1.0302±0.002^{b}	1.0303±0.002^{b}	1.0304±0.00^{2}b	1.0305±0.001^{b}	1.0303±0.00^{1}b	1.0299±0.00^{2}b	1.0305±0.0008^{b}
Acidity (°SH)	6.57±0.28^{a}	6.73±0.24^{b}	6.73±0.22^{b}	6.53±0.20^{a}	6.73±0.37^{b}	6.57±0.32^{a}	6.75±0.22^{b}
Fat (%)	4.05±0.69^{b}	4.00±0.43^{a,b}	4.10±0.36^{b}	4.05±0.54^{b}	3.80±0.60^{a}	4.00±0.58^{a,b}	4.05±0.33^{b}
Lactose (%)	4.52±0.26^{b}	4.36±0.17^{a}	4.50±0.21^{b}	4.43±0.24^{b}	4.38±0.17^{a}	4.36±0.20^{a}	4.35±0.19^{a}
Protein (%)	3.81±0.5^{7}a	3.95±0.4^{9}a	4.15±0.7^{4}b	3.72±0.3^{8}a	4.11±0.6^{2}b	4.03±0.55^{b}	4.14±0.43^{b}
SNF (%)	8.93±0.56^{a}	9.11±0.53^{b}	9.63±0.79^{c}	8.93±0.41^{a}	9.35±0.66^{b}	9.14±0.51^{b}	9.29±0.46^{b}
a_w	0.8995±0.02^{a}	0.9126±0.01^{b}	0.9032±.015^{a,b}	0.8958±0.01^{a}	0.9102±0.02^{b}	0.9008±0.01^{a,b}	0.9115±0.01^{b}
FP (°C)	-0.46±0.03^{a}	-0.47±0.02^{a}	-0.49±0.01^{a}	-0.48±0.02^{a}	-0.47±0.02^{a}	-0.48±0.02^{a}	-0.49±0.01^{a}
Conductivity-EC (mS/cm)	4.63±0.76^{a,b}	4.65±0.84^{a,b}	4.72±0.49^{b}	4.85±0.41^{b}	4.79±0.74^{b}	4.41±0.59^{a}	4.68±0.64^{a,b}
Ash (%)	0.83±0.06^{b}	0.76±0.08^{a,b}	0.74±0.05^{a}	0.71±0.10^{a}	0.74±0.03^{a}	0.73±0.06^{a}	0.80±0.04^{b}

Statistical analysis using one-way ANOVA followed by Tukey's HSD test ($p<0.05$) revealed statistically significant differences for most of the observed parameters between regions, as indicated by different lowercase letters (a, b, c, d) accompanying the mean values.

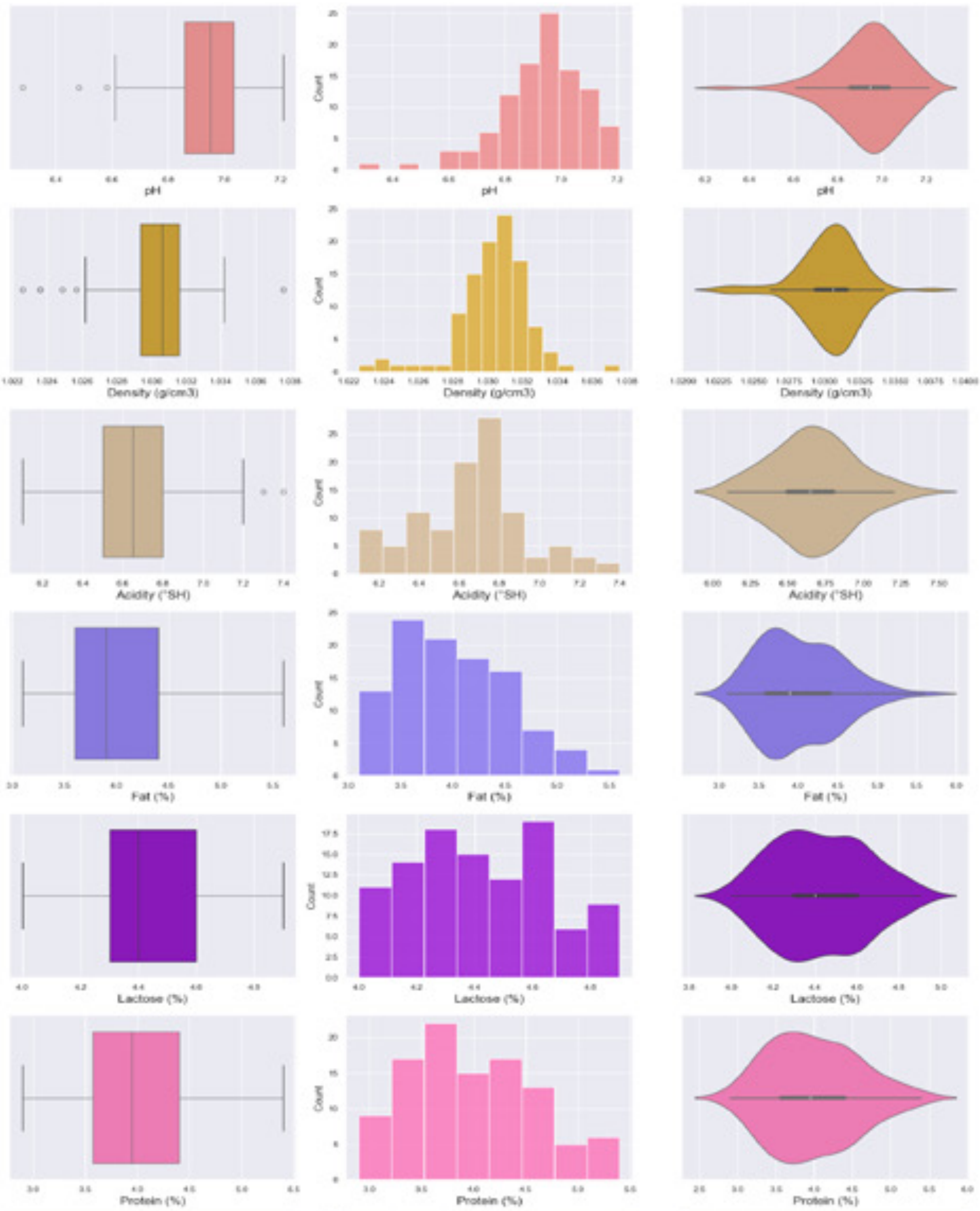

Figure 1. Distribution of pH, density, acidity, fat content, lactose content, and protein content in cow's milk, visualized using boxplots, histograms, and violin plots

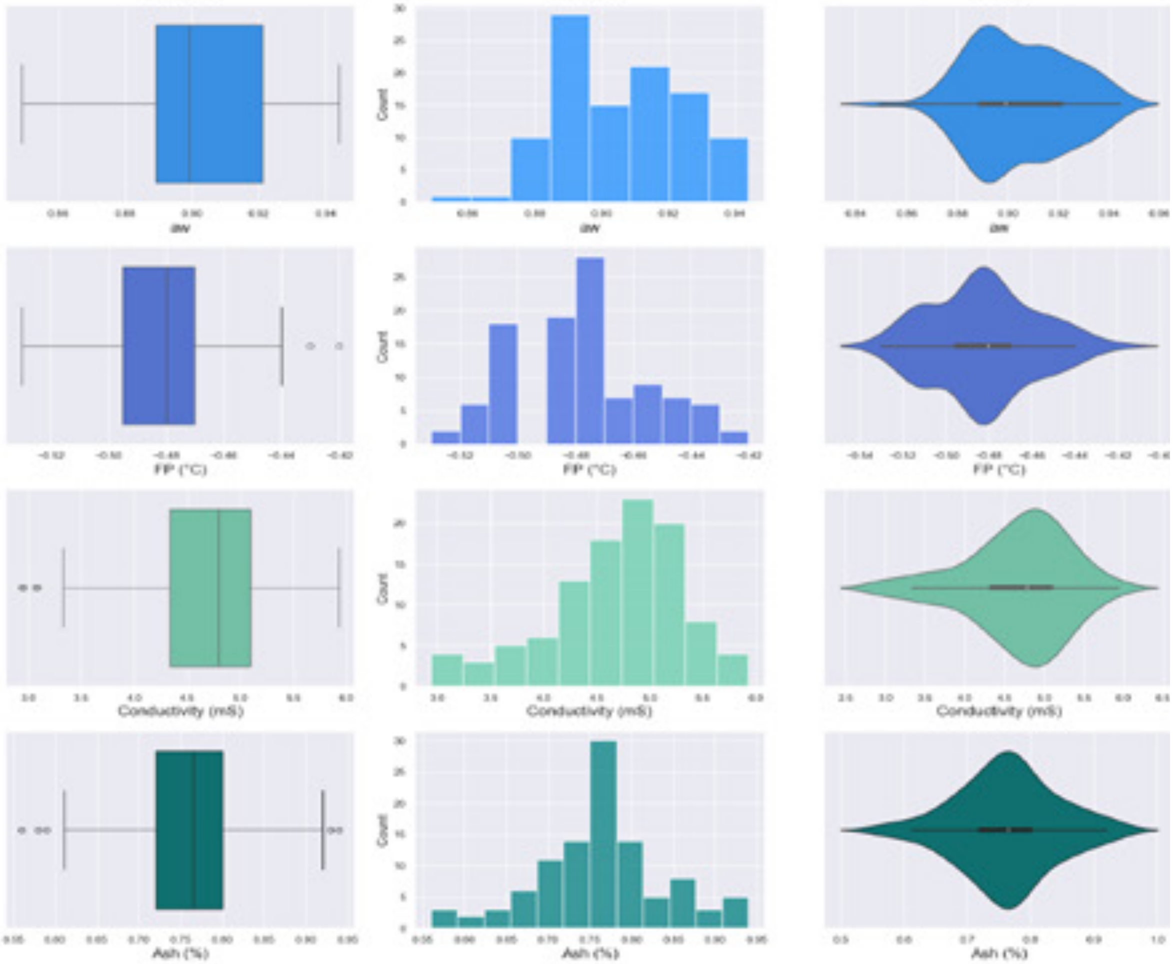

Figure 2. Distribution of solids-not-fat (SNF), water activity (a_w), freezing point (FP), conductivity, and ash content in cow milk samples, visualized using boxplots, histograms, and violin plots

Table 3. Mineral content of cow milk across different regions of Kosovo

Parameters	Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7
Ca (mg/L)	925.92± 153.35^{a}	1036.84± 134.13^{a}	1053.87± 210.18^{a}	933.67± 101.82^{a}	1078.07± 178.04^{b}	1053.05± 180.6^{b}	1104.37± 237.02^{b}
K (mg/L)	1361.01± 207.51^{b}	1418.69± 103.75^{c}	1378.23± 130.24^{b}	1404.63± 143.33^{b}	1267.82± 153.6^{a}	1431.99± 159.24^{b}	1395.95± 251.73^{b}
P (mg/L)	1433.7± 167.67^{b}	1307.23± 251.53^{a}	1598.27± 154.24^{c}	1400.55± 114.18^{b}	1401.64± 160.44^{b}	1375.09± $199.69^{a,b}$	1313.07± 298.03^{a}
Mg (mg/L)	74.49± 8.67^{a}	81.98± 6.55^{b}	81.67± 8.15^{b}	72.8± 10.71^{a}	79.59± 5.75^{a}	77.94± 9.74^{a}	80.05± $14.06^{a,b}$
Na (mg/L)	493.76± 142.84^{b}	534.93± 116.7^{c}	534.56± 128.99^{c}	496.58± 126.9^{b}	578.03± 125.52^{c}	442.83± 76.35^{a}	515.79± 144.79^{b}
Fe (mg/L)	1.09± 0.35^{a}	1.68± 0.91^{b}	0.91± 0.65^{a}	1.16± 0.87^{a}	0.78± 0.38^{a}	0.73± 0.68^{a}	1.46± 0.88^{b}
Zn (mg/L)	2.75± 0.88^{a}	3.76± 1.3^{b}	5.29± 1.64^{c}	2.71± 0.91^{a}	3.36± 0.69^{a}	3.41± 1.36^{a}	3.19± 1.06^{a}
Cu (mg/L)	2.04± 1.72^{a}	1.78± 1.58^{a}	1.65± 1.41^{a}	2.41± 2.4^{b}	2.28± 1.94^{a}	2.53± 2.36^{b}	2.07± 1.93^{a}
Mn (mg/L)	0.04± 0.02^{b}	0.03± 0.03^{a}	0.05± 0.02^{b}	0.04± 0.03^{b}	0.04± 0.02^{b}	0.05± 0.02^{b}	0.02± 0.01^{a}

Statistical analysis, performed using the one-way ANOVA with Tukey HSD ($p < 0.05$), showed statistically significant differences for most of the observed parameters between regions, which is indicated by different lowercase letters (a, b, c) along with the average values.

These regional differences in milk mineral composition are likely influenced by variations in soil mineral content, forage type, and feeding practices. Since plants absorb minerals directly from soil, geographical differences in soil composition can lead to significant variation in feed mineral content, however, this variation is not directly transferred to milk, due to homeostatic regulation of mineral secretion in the mammary gland (Gaucheron, 2005; Shennan and Peaker, 2000). This underscores the importance of considering environmental and agricultural factors when interpreting milk mineral profiles Castillo et al. (2013) examined mineral concentrations in diet, water, and milk across California dairies and reported that milk calcium, chlorine, and zinc were typically 10-20 % lower than NRC reference values, while sodium, copper, iron, and manganese often fell ≥36 % below. Our results similarly reveal sub-reference levels of calcium, magnesium, and zinc, indicating a comparable pattern of mineral under-transfer. Additionally, Castillo's model demonstrated that ignoring water minerals can distort feed-to-milk balance calculations - an aspect that should be addressed in future studies in Kosovo. Stergiadis et al. (2021) compared grazing and zero-grazing systems and found that grazing management significantly alters milk mineral composition, particularly increasing calcium, magnesium, and zinc in cows grazing mineral-rich pastures. Furthermore, a comparison of the mineral composition of raw milk across different countries is presented in table 4.

However, these findings should be interpreted with caution. Previous research (Toscano et al., 2023) indicates that mineral composition is more strongly associated with lactose content, whereas protein and density are influenced by broader dietary factors, including energy balance and overall nutrient composition (Alvarado et al., 2024). Thus, the associations observed in this study likely reflect indirect effects, where mineral-rich diets coincide with improved overall nutritional quality of feed, rather than a direct

Table 4. Mineral element concentrations in raw milk across countries in the region

Elements (mg/L)	Kosovo	North Macedonia	Croatia	FAO	Changes (mg/L) *
Ca	1026.2	1122	1403.94[a]	1120	-93.8
K	1384.14	1174	1582.1[b]	1450	-65.86
P	1414.89	786	930.6[b]	910	+504.89
Mg	78.45	80,8	165.01[a]	110	-31.55
Na	511.59	489	490.1[b]	420	+91.59
Fe	1.10	3,82	0.70[b]	1	+0.1
Zn	3.59	3.17	3.22[b]	4	-0.41
Cu	2.08	2,76	0.38[a]	Tr-Tr	+2.08
Mn	0.04	0.039	0.05[b]	0.08	-0.04
References	From this research	(Vllasaku et al., 2017)	(Sikirić et al., 2003; Vahčić et al., 2010) [a,b]	(FAO, 2013)	(–) denotes a value below the recommended amount (+) denotes a value above the recommended amount

**Source: Authors' calculations based on FAO data and our research.*

Table 5. Occurrence (%) and representation of animal feed types on cow farms in different regions of the Republic of Kosovo. The percentage represents the proportion of farms in each region that use a given type of feed

Feed type	Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7
Maize silage	85.7	78.6	75.0	60.0	90.9	56.3	92.9
Dry hay	92.9	92.9	90.0	100.0	100.0	93.8	92.9
Wheat bran	57.1	57.1	40.0	46.7	45.5	37.5	28.6
Granule concentrate (18 % protein)	57.1	78.6	85.0	100.0	36.4	56.3	85.7
Dry alfalfa	21.4	14.3	5.0	13.3	/	25.0	14.3
Ground corn	21.4	21.4	5.0	13.3	27.3	31.3	14.3
Vitamins and minerals	7.1	7.1	5.0	/	/	/	/
Dry oats	14.3	14.3	/	6.7	9.1	6.3	/
Alfalfa silage	7.1	7.1	/	6.7	/	/	21.4
Soybeans	/	7.1	/	/	/	/	/
Grass silage	/	/	65.0	46.7	/	/	42.9
Cranberry concentrate	/	/	5.0	/	/	/	/
Malt residues	/	/	15.0	/	/	/	/
Wheat straw	7.1	7.1	5.0	/	/	/	/
Corn silage	/	7.1	15.0	/	/	/	/
Dry wheat	/	7.1	/	/	/	/	/
Oat silage	/	/	5.0	/	/	/	7.1

causal relationship. This underscores the complexity of milk composition, where multiple dietary and physiological factors interact, and emphasizes the need for integrated multivariate approaches to better understand these relationships.

Finally, Visentin et al. (2018) used mid-infrared spectroscopy to characterize milk minerals from thousands of Italian samples, reporting wide farm-to-farm variation similar in magnitude to the inter-regional differences observed in Kosovo. Their findings confirm that such variability is intrinsic to dairy systems and can be efficiently captured using multivariate statistical methods. The application of multivariate analysis (MVA) is particularly important in milk quality studies, due to the complexity of compositional data (Mele et al., 2016; Correddu et al., 2021) and its relationship with metabolic and physiological factors than with direct dietary mineral intake, further supporting the concept of homeostatic regulation (Toscano et al., 2023). Therefore, the types of cow feed (table 5) and their mean compositions (table 6) were also analyzed.

Dominant components of the diet: Some ingredients are nearly universal, including dry hay, corn silage, concentrates, and bran. There are also region-specific differences: grass silage is highly prevalent in region 3 (65.0 %) and moderately common in regions 4 (46.7 %) and 7 (42.9 %), but it was not recorded in regions 1, 2, 5, or 6.

Table 6 presents the composition of animal feed used on cow farms in seven regions of the Republic of Kosovo, including regional mean values for protein, fat, ash, and concentrations of nine key minerals (mg/kg).

A more detailed regional interpretation highlights differences in feeding structure among Kosovo regions. For example, Region 3 is characterized by a higher proportion of grass silage, while Regions 4 and 7 show a greater reliance on concentrate-based feeding (Table 5). These differences are reflected in feed composition (Table 6), where higher concentrations of certain minerals and crude protein were observed in Regions 4 and 7. Correspondingly, milk from these regions exhibited higher SNF and protein content (Table 2). The higher proportion of concentrates in Regions 4 and 7 is likely associated with improved energy and protein supply, which may explain the observed differences in milk composition.

Although regional differences in the mineral composition of feed were identified (Table 6), their direct impact on milk mineral composition appears to be limited. This observation supports the concept of homeostatic regulation, whereby the mammary gland maintains a relatively constant mineral profile in milk despite variations in dietary mineral intake (Gaucheron, 2005; Shennan and Peaker, 2000). Similar findings were reported by Toscano et al. (2023), who demonstrated that variations in milk mineral composition are more closely associated with metabolic status and overall milk composition than with direct dietary mineral intake. To investigate the relationships between observed feed parameters and milk physicochemical characteristics, including macronutrient and mineral content, multivariate analysis using principal component analysis (PCA) was applied, and the results are presented in figure 3.

Multivariate principal component analysis (PCA) was applied to identify underlying structures and patterns in the data, and the biplot provides a visual and statistical representation of the clustering of regions and parameters based on compositional similarities for both milk and feed. The biplot is based on two principal components (axes) that explain the majority of the variance in the dataset: F1 (principal component 1) accounts for 32.89 % of the total variance, and F2 (principal component 2) accounts for 19.79 %. Together, these two components explain 52.68 % of the

Table 6. *Average protein, fat, and ash content, as well as mineral concentrations (mg/kg), in animal feed on farms in the Republic of Kosovo*

Parameters	Region 1	Region 2	Region 3	Region 4	Region 5	Region 6	Region 7
Proteins (%)	17.70±2.87^{b}	16.56±2.55^{a}	16.59±2.99^{a}	17.90±2.58^{b}	17.28±1.26^{b}	17.29±1.85^{b}	18.87±2.02^{c}
Fats (%)	2.29±0.92^{a}	2.21±0.82^{a}	2.55±0.95^{a}	2.03±0.95^{a}	2.82±0.89^{b}	2.15±0.90^{a}	2.11±0.58^{a}
Ash (%)	4.81±0.97^{a}	4.88±0.92^{a}	4.46±1.06^{a}	4.93±0.68^{a}	4.28±0.88^{a}	4.44±0.89^{a}	5.05±1.01^{a}
Ca (mg/kg)	4588.54± 2191.49^{a}	4966.01± 4692.38^{a}	5895.51± 2328.11^{b}	6537.12± 2532.53^{c}	3287.49± 1640.46^{a}	3683.85± 1133.05^{a}	5641.32± 3808.97^{b}
K (mg/kg)	9090.38± 1586.69^{b}	9500.08± 1662.42^{b}	9949.2± 2971.99^{b}	11036.7± 3039.64^{c}	7827.63± 2653.47^{a}	9434.18± 2671.59^{b}	9457.2± 2530.52^{b}
P (mg/kg)	7174.02± 1908.48^{b}	5265.5± 1374^{a}	5767.21± 2262.71^{a}	8131.46± 2758.47^{b}	6246.33± 2412.21^{b}	7060.82± 3033.16^{b}	6514.74± 1723.37^{b}
Mg (mg/kg)	1809.62± 678.76^{b}	1952.2± 1345.26^{b}	1661.43± 448.21^{a}	2127.67± 493.1^{b}	1863.92± 936.12^{b}	1394.41± 370.79^{a}	1857.05± 551.25^{b}
Na (mg/kg)	1719.99± 1570.86^{a}	1937.54± 2143.11^{a}	1846.76± 1069.51^{a}	1857.48± 555.53^{a}	1625.29± 871.88^{a}	1931.76± 1087.96^{a}	1566.07± 737.67^{a}
Fe (mg/kg)	327.08± 343.84^{a}	182.05± 129.91^{a}	294.88± 291.55^{a}	656.17± 1009.81^{a}	253.93± 545.87^{a}	200.2± 278.77^{a}	290.57± 223.47^{a}
Zn (mg/kg)	25.63± 18.64^{a}	46.87± 72.92^{b}	21.89± 20.22^{a}	36.58± 12.75^{b}	21.47± 11.65^{a}	31.33± 17.27^{a}	25.21± 17.19^{a}
Cu (mg/kg)	9.35± 5.68^{a}	16.37± 18.56^{b}	16.28± 8.07^{b}	10.54± 7.06^{a}	11.22± 7.39^{a}	8.74± 5.45^{a}	10.54± 7.97^{a}
Mn (mg/kg)	41.75± 17.02^{a}	54.35± 67.61^{a}	67.99± 66.61^{a}	83.87± 41.85^{b}	39.06± 24.96^{a}	43.93± 19.46^{a}	47.29± 20.16^{a}

Statistical analysis, performed using the one-way ANOVA with Tukey HSD ($p < 0.05$), showed statistically significant differences for most of the observed parameters between regions, which is indicated by different lowercase letters (a, b, c) along with the average values.

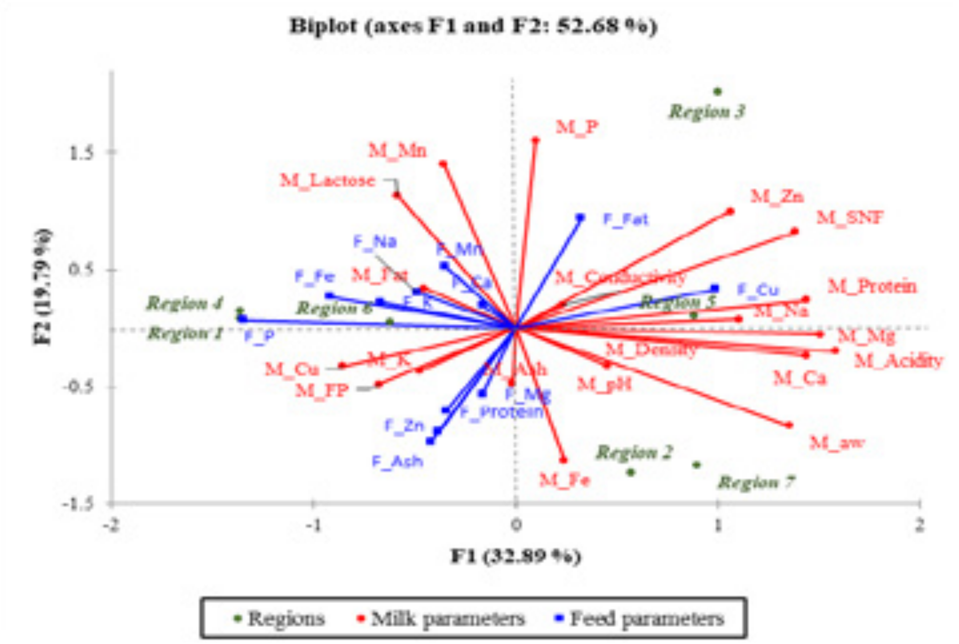

Figure 3. *Biplot showing relationships between milk parameters and cows' feed composition (M: milk; F: feed)*

total variance in the observed milk and feed parameters. Parameters grouped within the same quadrant indicate a dominant relationship between them (Correddu et al., 2021; Ithurbide et al., 2023). The PCA results provide important insights into the relationships between milk composition and feed characteristics across regions. Clear clustering of regions was observed, reflecting differences in both milk composition and feeding practices. For example, regions 2 and 7 were associated with higher water activity and iron content in milk, whereas regions 3 and 5 were characterized by higher fat and copper content in feed. A strong positive association was observed between calcium and magnesium in feed and milk density, as indicated by Pearson correlation coefficients (r_{Ca} = 0.787; r_{Mg} = 0.767). This association should not be interpreted as a direct causal effect. Instead, it likely reflects co-variation within feeding systems, where mineral-rich feed is associated with improved overall nutritional balance (energy, protein, and digestibility), which indirectly supports milk synthesis (Toscano et al., 2023). However, this relationship is likely indirect and reflects overall dietary quality rather than a direct causal effect. The observed associations, particularly the link between calcium and magnesium with milk density and their clustering alongside protein-related parameters in the PCA, suggest a potential connection between mineral availability and overall milk composition. This is further supported by strong correlations in our dataset, where milk calcium and phosphorus clustered closely with SNF and protein loadings in PCA space, highlighting the shared biochemical mechanisms of casein micelle formation and mineral binding. These findings reinforce our PCA-based observation that regions with feed richer in these minerals (e.g., regions 4 and 7) produced milk with higher SNF and protein supporting the observed associations between feeding regime and milk quality parameters. This suggests that the feeding regime and forage composition are associated with variations in milk quality, primarily through indirect physiological mechanisms.

The influence of dietary minerals on milk mineral composition is generally moderate under normal feeding conditions. Significant deviations may occur only in cases of severe mineral imbalance, such as hypocalcaemia, inappropriate Ca:P ratios, or excessive supplementation. In such cases, the effects are more often expressed through altered animal health, metabolic status, or increased risk of mastitis, rather than direct changes in the basic mineral profile of milk.

Overall, the PCA highlights that regional variation in milk composition is closely linked to differences in feed composition, underscoring the importance of diet as a key driver of milk quality. This approach enables the identification of dominant modes of variation within a set of functional data. Integrating milk and feed data within the same analytical framework represents a key strength of this study, providing a more comprehensive understanding of nutrient interactions and regional variability (Harwood and Drake, 2020; Tangorra et al., 2022). However, it is important to note that Principal Component Analysis (PCA) has limitations in interpretation compared to Multiple Factor Analysis (MFA). The study by Davis et al. (2020) confirmed the ability to group fatty acids according to function or metabolic origin. Nevertheless, the first principal component (PC1) often exhibits structural similarity to the first latent factor (F1) obtained by MFA, both of which are associated with dairy-origin fatty acids (Harwood and Drake, 2020).

The results of the principal component analysis (PCA) further support this interpretation. The observed associations between feed characteristics and milk composition should not be interpreted as direct causal relationships. Rather, they likely reflect co-variation within feeding systems, where mineral-rich feed is associated with improved overall nutritional balance, including energy and protein supply, which indirectly supports milk synthesis. This interpretation is consistent with previous findings indicating that milk composition is more strongly influenced by the overall nutritional and metabolic status of the animal than by individual dietary components (Toscano et al., 2023).

The influence of dietary minerals on milk composition is therefore generally moderate under normal feeding conditions. More pronounced effects may occur only in cases of severe mineral imbalance, such as hypocalcaemia, inappropriate Ca:P ratios, or excessive supplementation. In such situations, the effects are more commonly expressed through alterations in animal health, metabolic status, and increased susceptibility to mastitis, rather than through direct changes in the basic mineral profile of milk.

Overall, the results of this study indicate that regional differences in milk composition in Kosovo are primarily associated with variations in feeding systems and overall diet quality. However, these effects should be understood as indirect and mediated through animal physiology, rather than as a direct transfer of minerals from feed to milk.

Conclusions

This study provides a comprehensive assessment of regional variability in the physicochemical and mineral composition of cow's milk and animal feed across seven regions of Kosovo, using a multivariate statistical approach. The results confirmed statistically significant regional differences in most analyzed milk parameters, reflecting the heterogeneity of dairy production systems and feeding practices across the country. Milk samples from Kosovo were characterized by higher average concentrations of phosphorus (P) and sodium (Na) compared to international reference values, while calcium (Ca), potassium (K), magnesium (Mg), zinc (Zn), and manganese (Mn) were generally lower. At the same time, substantial regional variation in feed composition was observed, including differences in both macro- and micromineral content as well as feeding strategies. However, the interpretation of these relationships must consider the physiological regulation of milk synthesis. The mineral composition of milk is largely controlled by homeostatic mechanisms in the mammary gland, which maintain relatively stable concentrations of key minerals across a wide range of dietary conditions. Therefore, the observed associations between feed composition and milk mineral profile should be interpreted as indirect and indicative of overall nutritional and management conditions rather than as evidence of direct mineral transfer from feed to milk. Multivariate analysis, particularly principal component analysis (PCA), proved useful for identifying patterns of regional clustering and associations between milk and feed parameters. However, these relationships represent statistical co-variation and should not be interpreted as causal. The results highlight that regional differences in milk composition are more closely linked to the overall structure and balance of the diet, as well as farm management practices, than individual mineral concentrations alone. From a practical perspective, improving feed formulation and maintaining balanced nutrition may contribute to enhanced milk quality, particularly in terms of physicochemical properties such as protein and solids-not-fat content. Nevertheless, targeted modification of the mineral profile of milk through dietary manipulation alone is likely to have limited effectiveness under normal physiological conditions due to strong homeostatic regulation.

Overall, this study provides a valuable basis for understanding regional variability in Kosovo's dairy sector and supports the development of region-specific strategies for improving milk quality. Future research should include controlled feeding experiments, seasonal analyses, and the incorporation of additional factors such as water mineral composition and animal health indicators in order to better quantify nutrient transfer mechanisms and validate the observed associations.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contributions:

Conceptualization. I.L., V.K., A.E., S.M., V.S. and J.G.K.; methodology. I.L., V.K., A.H., A.E., S.M., V.S. and J.G.K.; software. V.K. and J.G.K.; validation. I.L., V.K., A.E., S.M., V.S. and J.G.K.; formal analysis. I.L., V.K. and J.G.K.; investigation. I.L., V.K., A.E., S.M., V.S. and J.G.K.; resources. I.L., V.K., A.E., S.M., V.S. and J.G.K.; data curation. I.L., V.K., A.E., S.M., V.S. and J.G.K.; writing-original draft preparation. I.L., V.K. and J.G.K.; writing-review and editing. I.L., V.K., A.E., S.M., V.S., A.H., and J.G.K.; visualization. V.K. and J.G.K.; supervision. A.E. and V.K.; project administration. I.L.; funding acquisition. J.G.K. All authors have read and agreed to the published version of the manuscript.

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Multivarijatna statistička procjena regionalnih razlika u mineralnom sastavu kravljeg mlijeka i stočne hrane na Kosovu

Sažetak

Ovo istraživanje ispitivalo je regionalne razlike u sastavu sirovog kravljeg mlijeka i stočne hrane s ciljem utvrđivanja njihove međusobne povezanosti primjenom multivarijatne analize. Istraživanjem su obuhvaćene 104 farme za proizvodnju sirovog kravljeg mlijeka (s više od 10 muznih krava) u sedam regija Kosova tijekom razdoblja 2022.–2024. godine. Analizirani su fizikalno-kemijski parametri mlijeka (pH, gustoća, kiselost, mast, laktoza, proteini, a_w, bezmasna suha tvar (SNF), vodljivost, FP i pepeo), dok su makroelementi (Ca, K, P, Mg, Na) i mikroelementi (Fe, Zn, Cu, Mn) određivani u mlijeku i stočnoj hrani. Statistička analiza uključivala je deskriptivnu statistiku i jednosmjernu ANOVA analizu (s Tukey HSD post-hoc testom) radi utvrđivanja regionalnih razlika. Analiza glavnih komponenti (PCA) primijenjena je za istraživanje multivarijatnih učinaka i odnosa između parametara mlijeka i hrane. Statistički značajne regionalne razlike potvrđene su kod većine promatranih fizikalno-kemijskih i mineralnih parametara sirovog kravljeg mlijeka. Prosječan mineralni sastav mlijeka s kosovskih farmi pokazao je više koncentracije fosfora (P) i natrija (Na) u odnosu na međunarodno preporučene vrijednosti (FAO), dok su koncentracije Ca, K, Mg, Zn i Mn bile niže od preporučenih vrijednosti. Uočena je pozitivna korelacija između Mg i Ca u stočnoj hrani te gustoće mlijeka, što je potvrđeno Pearsonovim koeficijentima korelacije r_{Ca} = 0,787 i r_{Mg} = 0,767. Primjena multivarijatne analize (MVA) bila je iznimno važna za dobivanje integrirane procjene prijenosa hranjivih tvari i regionalne diferencijacije. Općenito, rezultati ukazuju na to da bi poboljšanje formulacije hrane i mineralne ravnoteže moglo unaprijediti nutritivnu kvalitetu mlijeka, pružiti znanstvenu osnovu za regionalno brendiranje mliječnih proizvoda te promicati održivo upravljanje stočarskom proizvodnjom.

Ključne riječi: kravlje mlijeko; stočna hrana; kvaliteta mlijeka; multivarijatna analiza; mineralni sastav

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