



Exploring the Effects of Altitude on Milk Composition: Insights from Kosovo's Diverse Localities

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ABSTRACT

Background: This study examines how altitude affects the physicochemical characteristics of milk by examining samples taken from three different altitude ranges in Kosovo: low altitude (below 600 m), medium altitude (600-800 m) and high altitude (above 800 m). Identification of changes in milk's physical and chemical properties brought on by altitude variations was the goal.

Methods: Between April and June 2021-2023, 85 farmers in 13 municipalities in Kosovo provided samples, which were then examined for factors such as fat, protein, casein, SNF, TS, density, freezing point, acidity (Dornik, Soxhlet Henkel, Thörner), lactose, galactose, glucose, organic acids and urea. In compliance with ISO 9622:2013 and AOAC 972.16 standards, measurements were made using the MilkoScan FT device (FOSS, Hilleroed, Denmark) with accuracy < 1% CV and reproducibility ≤0.25% CV. SPSS was used to conduct statistical analysis, evaluating height group differences using the LSD test and ANOVA.

Result: Differences in freezing point, lactose, galactose, glucose and lactic acid were statistically significant ($p < 0.05$). The freezing point was lower and the quantities of lactose, galactose and glucose were higher at high altitudes, indicating a strong correlation between the two variables. However, no significant differences ($p > 0.05$) were found for urea, fat, protein, casein, solids non-fat (SNF), total solids (TS), citric acid, or free fatty acids (FFA). This suggests that altitude has less of an impact on these parameters than genetics and animal food.

Key words: Altitude effect, Dairy farming, Geographic variation, Kosovo localities, Milk quality assessment, Physicochemical properties.

INTRODUCTION

The Republic of Kosovo covers 10,905.25 km² in southeastern Europe, bordered by Albania, Montenegro, Serbia and North Macedonia. Its terrain ranges from 270 m near the Drini i Bardhë river to 2,656 m at Gjeravicë (MAFRD 2021). Kosovo has a continental climate, with temperatures varying from + 30°C in summer to -10°C in winter (MAFRD 2021). Agriculture is a cornerstone of the economy (MAFRD 2023), with 420,482 ha of agricultural land in use in 2022 (MAFRD 2023). Milk production is a key income source for farmers (Mestani *et al.*, 2023). In 2022, Kosovo had 250,899 cattle, 52% being dairy cows, producing 276 thousand tons of milk-a 1% decrease from 2021, primarily due to fewer dairy cows. Of this, 81% was domestically produced and the rest imported (MAFRD, 2023).

Milk composition depends on intrinsic factors like genetics, breed, somatic cell count, lactation stage and extrinsic factors such as climatic environment and feeding practices (Li *et al.*, 2014; Hofstetter *et al.*, 2014; Kumar and Mandal 2023; O'Callaghan *et al.*, 2016; Stocco *et al.*, 2017; Shah *et al.*, 2024; Stadnik and Atasever, 2016). Altitude significantly affects milk composition in mountainous regions, influencing atmospheric pressure, temperature, solar radiation and feed availability (Quiao *et al.*, 2013; Neary *et al.*, 2013). These factors interact with dairy cows' physiology, altering milk quality (Quiao *et al.*, 2013; Neary *et al.*, 2015; Saha *et al.*, 2019; Kong *et al.*, 2021; Andjelić *et al.*, 2022).

Research shows altitude impacts milk composition. Saha *et al.* (2019) found higher fat content in milk from

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cows at alpine pastures above 1,200 m compared to lower altitudes. Similarly, Zemp *et al.* (1989) observed higher fat but lower protein and lactose levels at high altitudes. Alrhoun *et al.* (2023) reported increased fat, protein, urea and somatic cell counts in milk from high-altitude farms. These differences arise from factors like feed intake, breed adaptations and physiological changes at high altitudes (Neary *et al.*, 2013; Zendri *et al.*, 2016; Churakov *et al.*, 2021).

Variations in management practices, such as housing and feeding regimes (Popescu *et al.*, 2014) and environmental stressors further affect milk quality (Costa *et al.*, 2019; Friedrich and Wiener 2020). For example, alpine forage with high crude fiber content can influence nutrient digestibility and milk protein content (Pittarello *et al.*, 2018; Pornaro *et al.*, 2019; Ineichen *et al.*, 2019; Zhang *et al.*, 2022). While studies on altitude's effects on milk composition in camels, goats and sheep are extensive, research on dairy cows remains limited (Mohammed *et al.*, 2022), particularly in mountainous areas (Battaglini *et al.*, 2014). Bytyqi *et al.* (2009) explored milk production in Kosovo but provided limited insights into altitude's impact on milk characteristics. Similarly, Mestani *et al.* (2017a; 2017b) and Mehmeti *et al.* (2016) analyzed milk composition, including its physicochemical and microbiological properties, across different locations in Kosovo but did not emphasize the influence of altitude. This study addresses this gap by examining the effects of altitude on milk physicochemical characteristics across low, medium and high altitudes in Kosovo.

MATERIALS AND METHODS

The study samples

Samples of cow's milk were gathered from 85 farmers spread over 13 municipalities in Kosovo, ranging in elevation from low to high. A variety of parameters, including the size of the municipality, altitude, number of farmers and the heterogeneity of milk qualities, were taken into consideration while determining the number of samples. Farmers were chosen at random from various regions to provide sufficient participation. The samples, which were taken in April and June of 2021, 2022 and 2023, were treated

with sodium azide (0.04%) to stop the growth of bacteria. The samples were divided into three altitude categories: low (below 600 m), medium (600-800 m) and high (above 800 m). The farms from which the samples were taken are indicated on the blue-starred map of Kosovo (Fig 1), which also shows the distribution of the farms and the effects of altitude on milk quality.

Physiochemical characteristics analyses

The physio-chemical analysis of milk samples was conducted using the MilkoScan FT device (FOSS, Hilleroed, Denmark). The device offers ready-made calibrations supported by the FOSS database, ensuring compatibility with chemical methods and compliance with ISO 9622:2013/IDF 141:2013 and AOAC official method 972.16. The parameters analyzed include Fat (%), Protein (%), Casein (%), SNF (solids not fat) (%), TS (total solids) (%), Density (g/cm³), Freezing Point (°C), Dornic Acidity (°D), Soxhlet Henkel Acidity (°SH), Thörner Acidity (°Th), Lactose, Low Lactose (%), Galactose (%), Glucose (%), Lactic Acid (%), Citric Acid (%), FFA (Free Fatty Acids) (mmol/kg) and Urea (mg/100 ml, mg/dL, or mg/L). The device's accuracy is ≤1% CV and reproducibility is ≤0.25% CV, ensuring precise and reliable results for milk quality control in line with international standards. The analyses were carried out in triplicate at the laboratories of the Food and Veterinary Agency in Kosovo.

Population statistics and altitude measurement

Population statistics were obtained from the Statistical Agency of Kosovo (SAK), using estimated data by municipality from 2012 to 2021 (SAK 2022). Geographical data, including altitude and municipality locations, was sourced from the Elevation API. World topographic maps



Fig 1: Map of Kosovo with blue stars indicating sample collection locations.

were also consulted for accuracy (^bElevation data in JSON, GeoTIFF and KML). For further details, visit: <https://en-gb.topographic-map.com/>.

Statistical analysis

Descriptive statistics summarized the characteristics of the data, while inferential statistics, specifically the ANOVA test, were conducted using SPSS-IBM Version 28.0, Armonk, NY. A p-value of 0.05 was considered statistically significant. After ANOVA, the LSD (Least Significant Difference) test was used to identify specific variations in the mean values of the parameters based on altitude.

RESULTS AND DISCUSSION

This section presents the findings of the study on the impact of altitude on the physio-chemical characteristics of milk across various municipalities in Kosovo.

Geographic overview and sample characteristics

The altitudes vary significantly, ranging from 440 meters in Klinë to 1,092 meters in Pejë, indicating a diverse geographic landscape that may influence dairy farming practices and milk composition (Table 1).

Analysis of milk composition

Table 2 shows the results of various physical and chemical parameters analyzed by milk samples taken at various altitude. For the parameters that have statistically significant differences ($p < 0.05$), there are clear variations between altitudes (Table 2). The freezing point ($^{\circ}\text{C}$) of milk varies significantly between high and low altitudes ($p = 0.012$), indicating that altitude affects this temperature. Likewise, there are notable variations in the percentages of lactose (%), galactose (%) and glucose (%) at high and low altitudes ($p = 0.008$, $p = 0.006$ and $p = 0.029$, respectively), with higher percentages at higher altitudes.

A significant difference between high and medium altitudes is also seen in galactose (%), with higher levels of galactose at medium altitudes than at low altitudes ($p = 0.046$). This finding raises the possibility that the percentages of sugars and the freezing point of milk are related. Since soluble solids have an impact on freezing temperature, increasing the percentage of sugars in milk may have a similar effect by lowering the freezing temperature at higher elevations where sugar levels are higher. Additionally, there are notable variations in lactic acid (%) between high and medium altitudes ($p = 0.005$) and between high and low altitudes ($p = 0.039$), suggesting that altitude affects the amount of lactic acid in milk. A few milk components, however, do not exhibit statistically significant differences ($p > 0.05$) across altitudes, indicating that altitude has less of an impact on these parameters than other factors like animal genetics or feed management (Table 2). Because the p values are greater than 0.05, fat, protein, casein, SNF and TS do not exhibit statistically significant differences, indicating that altitude variations have no discernible effect on these components. Additionally, density does not significantly differ between groups. Likewise, citric acid, FFA and urea do not show statistically significant differences between altitudes ($p > 0.05$), suggesting that altitude does not have a significant impact on these parameters.

Last but not least, Table 3 it gives two important details for each parameter: the effect's magnitude and its significance level. The "Magnitude" column indicates the strength of altitude's effect, with positive values suggesting an increase and negative values indicating a decrease. The "Significance" column presents p-values that assess the statistical significance of the observed effects. Parameters with larger magnitudes and smaller p-values (e.g., Freezing Point, Lactose, Galactose) suggest that

Table 1: Population, Altitudes and Coordinates of Municipalities in Kosovo.

^a Municipality	^a Population	^b Altitude m (average elevation)	^b The coordinates (Topographic maps) Latitude and longitude coordinates
Dragash	33,482	1,060	42.05053 20.62007 42.07432 20.65942
Ferizaj	105,663	578	42.35615 21.11425 42.38754 21.18856
Fushë Kosovë	39,257	551	42.61107 21.06645 42.66174 21.12219
Gjakovë	92,827	543	42.21988 20.27162 42.53988 20.59162
Gjilan	73,518	531	42.43513 21.43739 42.48618 21.49648
Istog	41,119	735	42.74168 20.44825 42.82168 20.52825
Klinë	40,175	440	42.57799 20.53611 42.65799 20.61611
Lipjan	57,457	554	42.50674 21.09420 42.54295 21.15034
Mitrovica	68,238	788	42.71904 20.70579 43.03904 21.02579
Pejë	97,271	1,092	42.49940 20.12858 42.81940 20.44858
Prizren	193,806	914	42.05302 20.57633 42.37302 20.89633
Rahovec	55,391	521	42.35987 20.61274 42.43987 20.69274
Skenderaj	52,586	668	42.70757 20.74916 42.78757 20.82916

^aThe statistical agency of Kosovo (ASK): Estimated data by municipality, 2012-2022. <https://ask.rks-gov.net/>.

^bElevation data in JSON, GeoTIFF and KML formats using Elevation API. <https://en-gb.topographic-map.com/>.

altitude has a more substantial and statistically significant effect on these components.

Overall, the findings in Table 2 and 3 demonstrate that while some milk parameters-particularly those pertaining to sugars, acidity and freezing point-variate considerably with altitude, other parameters stay largely constant and are unaffected by variations in altitude.

The discussion compares the study's findings with existing literature, revealing that some milk parameters changed significantly with altitude, while others did not. The freezing point, for example, was lower at higher altitudes ($p<0.05$), which may be influenced by changes in milk's chemical composition. A negative correlation was found between freezing point and lactose, glucose and galactose levels ($p<0.05$), where lower temperatures were associated with higher sugar levels. Previous studies indicate that the freezing point can be affected by factors such as water addition, formaldehyde, antibiotics, sodium carbonate, hydrogen peroxide, disinfectant and detergent (Zagorska and Ciprovica, 2013). Our study found that lactose content was higher at high altitudes compared to medium and low altitudes, with significant differences between high and low altitudes ($p<0.05$). This contrasts with Saha *et al.* (2019) and Costa *et al.* (2019), who reported lower lactose content at higher altitudes, while Leiber *et al.* (2006) highlighted the role of forage origin and diet. Factors like feed composition and rumen metabolism may indirectly affect galactose and glucose levels (Zhang *et al.*, 2022; Getahun *et al.*, 2019), though altitude's direct impact remains unclear. Significant differences were observed in

acidity parameters (Dornic, Soxhlet Henkel, Thörner) between altitude groups ($p<0.05$), with lactic acid showing a notable variation ($p<0.05$) while citric acid did not ($p>0.05$). This suggests that lactic acid is more influenced by altitude, while citric acid levels may be less affected by these environmental factors. Other studies indicate that feed composition and rumen metabolism can influence these acids (Zhang *et al.*, 2022; Getahun *et al.*, 2019), but their relationship with altitude is unexplored. Furthermore, the correlations between blood and milk parameters, as investigated by Andjelica *et al.* (2022), may influence lactic acid and citric acid levels; however, their relationship with altitude remains unexplored. The study also found a positive correlation between lactic acid and several sugars ($p<0.05$), suggesting that higher sugar levels may contribute to increased lactic acid production. Fat percentage did not show significant differences between altitude groups ($p>0.05$). However, Saha *et al.* (2019) and Alrhoun *et al.* (2023) reported higher fat content in cows at higher altitudes. This suggests that factors beyond altitude, such as breed composition, feeding practices and environmental conditions, may affect milk fat content. Protein and casein percentages did not show significant differences ($p>0.05$), aligning with Qiao *et al.* (2013) and Abraham and Gayathri (2015), who found that these parameters are more influenced by feed and breed than altitude. Solid components (SNF, TS), density, FFA and urea also showed no significant differences ($p>0.05$), supporting findings by Qiao *et al.* (2013) and Abraham and Gayathri (2015), who argued that altitude has a limited effect on

Table 2: Physico-chemical parameters of milk (mean $\pm$ SD) across altitudes in Kosovo with pairwise comparison p-values.

Parameter	High altitude (above 800 m)	Medium altitude (600 m to 800 m)	Low altitude (below 600 m)	High vs Medium p-value	High vs Low p-value	Medium vs Low p-value
Fat (%)	3.77 $\pm$ 0.68	3.71 $\pm$ 0.98	3.79 $\pm$ 1.63	0.899	0.977	0.769
Protein (%)	3.22 $\pm$ 0.27	3.19 $\pm$ 0.26	3.22 $\pm$ 0.41	0.646	0.986	0.653
Casein (%)	2.46 $\pm$ 0.23	2.4 $\pm$ 0.3	2.46 $\pm$ 0.3	0.127	1.000	0.065
SNF (%)	8.58 $\pm$ 0.4	8.45 $\pm$ 0.54	8.49 $\pm$ 0.77	0.153	0.206	0.818
TS (%)	12.34 $\pm$ 0.84	12.18 $\pm$ 1.07	12.28 $\pm$ 1.79	0.572	0.873	0.756
Density (g/cm ³)	1028.06 $\pm$ 1.59	1027.66 $\pm$ 2.24	1026.95 $\pm$ 4.38	0.997	0.537	0.660
Freezing point (°C)	-0.53 $\pm$ 0.02	-0.52 $\pm$ 0.02	-0.52 $\pm$ 0.05	0.417	0.012	0.510
Dornic acidity (°D)	14.61 $\pm$ 1.17	14.1 $\pm$ 1.66	14.3 $\pm$ 1.58	0.005	0.038	0.339
Soxhlet henkel Ac. (°SH)	6.49 $\pm$ 0.6	6.23 $\pm$ 0.91	6.35 $\pm$ 0.74	0.003	0.075	0.163
Thörner acidity (°Th)	16.22 $\pm$ 1.25	15.68 $\pm$ 1.72	15.88 $\pm$ 1.73	0.006	0.038	0.381
Lactose (%)	4.6 $\pm$ 0.34	4.5 $\pm$ 0.42	4.48 $\pm$ 0.52	0.128	0.008	0.894
Galactose (%)	2.39 $\pm$ 0.08	2.31 $\pm$ 0.1	2.29 $\pm$ 0.14	0.046	0.006	1.000
Glucose (%)	2.10 $\pm$ 0.11	2.05 $\pm$ 0.16	2.01 $\pm$ 0.2	0.734	0.029	1.000
Lactic acid (%)	0.15 $\pm$ 0.01	0.14 $\pm$ 0.02	0.14 $\pm$ 0.02	0.005	0.039	0.336
Citric acid (%)	0.16 $\pm$ 0.03	0.16 $\pm$ 0.03	0.15 $\pm$ 0.03	0.907	0.120	0.063
FFA (mmol/kg)	0.45 $\pm$ 0.35	0.37 $\pm$ 0.74	0.42 $\pm$ 0.19	0.112	0.651	0.288
Urea (mg/100 mL)	25.77 $\pm$ 7.47	24.27 $\pm$ 7.63	24.01 $\pm$ 10.04	0.280	0.057	0.952
Urea (mg/L)	257.71 $\pm$ 74.72	242.72 $\pm$ 76.27	240.14 $\pm$ 90.39	0.280	0.057	0.952

Table 3: Altitude's influence on milk composition parameters via multivariate analysis.

Parameter	Magnitude	Significance
Fat (%)	-0.011	0.734
Protein (%)	-0.001	0.985
Casein (%)	-0.015	0.656
SNF- solids not fat (%)	0.051	0.125
TS- total solid (%)	0.012	0.732
Density g/cm ³	0.041	0.225
Freezing point (°C)	-0.100	0.003
Dornic acidity (°D)	0.070	0.036
Soxhlet henkel acidity (°SH)	0.059	0.044
Thörner acidity (°Th)	0.071	0.035
Lactose	0.077	0.022
Galactose (%)	-0.101	0.003
Glucose (%)	0.048	0.049
Lactic acid (%)	0.070	0.037
Citric acid (%)	0.079	0.118
FFA (mmol/kg)	0.019	0.575
Urea- mg 100 ml (mg/dL)	0.077	0.222
Urea-mg/L (mg/L)	0.077	0.122

these components. Our study observed higher FFA levels at higher altitudes, but the differences were not statistically significant ($p>0.05$). Lu *et al.* (2021) found lower FFA content during summer, suggesting that seasonal factors may be more influential than altitude. Urea levels were slightly higher at higher altitudes, but the differences were not statistically significant ($p>0.05$), indicating that factors like feed quality and rumen microbial activity (Zhang *et al.*, 2022; Getahun *et al.*, 2019) may have a stronger influence on urea concentrations.

Study limitations

The study focuses on physio-chemical characteristics of milk but does not address factors like cow breed, feeding practices, milking techniques and long-term altitude effects. Its limited sample size and geographical scope in Kosovo may restrict the generalizability of findings. The study also overlooks variations in pasture characteristics, weather conditions and milk handling practices, such as sanitation, udder washing and temperature maintenance, which could impact milk quality. Additionally, it does not explore the effects of storage and transportation methods on milk characteristics.

CONCLUSION

Significant variations were noted in the freezing point and sugar content, which have a strong correlation with one another. Acidity levels and lactic acid content may have changed as a result of the variations in sugar content. In particular, there were notable variations in freezing point between high and low altitudes ($p<0.05$), with higher altitudes showing a decrease in freezing temperature,

which was linked to higher sugar percentages. Similarly, at higher altitudes compared to lower altitudes, levels of lactic acid and acidity increased with higher sugar content ($p<0.05$). Changes in parameters like frizing point, lactose, galactose, glucose, acidity and lactic acid ($p<0.05$) show that altitude significantly affects these physicochemical characteristics. However, there were no significant differences ($p>0.05$) in the levels of other milk components such as fat, protein, casein, solids-not-fat (SNF), total solids (TS), density, free fatty acids (FFA) and urea at different altitude. Altitude appears to selectively influence specific physicochemical properties of milk, likely driven by other factors such as animal diet, genetics, climate, vegetation and farming practices. To clarify these mechanisms and evaluate their effects on milk quality and dairy production, more research is required.

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Data availability statement

Data are available upon reasonable, by the Corresponding Authors.

Conflicts of interest

The authors declare no conflict of interest.

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