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Influence of altitude and lactation period on composition and physical properties of milk in crossbred Sharri sheep

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Abstract. The aim of this study was to compare the changes in chemical composition and physical properties of raw milk from crossbred 'Sharri' sheep as a function of altitude (1,200 m - variant A and 1,600 m - variant B) and lactation period. Milk composition (total solids - TS, milk fat–MF, solids non-fat-SNF, protein - P, lactose - L, ash, freezing point - FP, titratable acidity - TA, active acidity - AA, and density - D) were analysed once per month during the period of June, July, August, and September of 2021. Sheep milk at 1,600 m altitude showed significantly higher values ($P < 0.05$) for all analysed parameters and lower values for FP compared to milk from 1,200 m altitude, except SNF, FP and AA, which were not statistically significant. The analysed milk during the four months of lactation period has significantly increased ($P < 0.05$) in TS (from 15.65 to 25.46%); MF (6.49 to 10.80%); SNF (9.16 to 14.66%); P (4.46 to 6.94%); L (3.96 to 6.52%); ash (0.74 to 1.20%); TA (9.90 to 14.53 °SH); whereas changes in FP value (-0.611 to -0.709 °C), AA (6.68 to 6.76) and D (1.035 to 1.053 g cm^{-3}), have been statistically non-significant. The interactions between altitude*lactation period on milk composition (TS, MF, SNF, P, L, ash) of crossbred 'Sharri' sheep and physical properties (FP, TA, AA and D) were nonsignificant.

Key words: altitude, crossbred 'Sharri' sheep, lactation period, milk composition, physical properties.

INTRODUCTION

Sheep milk represents an animal food product with high nutritional value. Its composition consists of hundreds of different components necessary for the normal development of the organism at each stage of life (Stojanović & Katić, 1998). According to Martini et al. (2008), interest in the nutrient composition of sheep milk has increased due to the nutritional value of milk in human diets. Nguyen (2022), stated that there is a good opportunity for the development of the sheep milk industry by virtue of its good nutritional value. In addition, Balthazar et al. (2017) emphasized that the physicochemical and nutritional characteristics of sheep milk can be beneficial for the manufacture of products with prebiotic ingredients and/or probiotic bacteria, which are important categories in the functional food market. The composition and physical properties of milk vary over time among animals depending on several factors such as the origin of the milk, breeding and genetics, health, age and size of the lactating animal, environment, nutrition and lactation period (Vujičić, 1985; Morand-Fehr et al., 2007; Ceyhan et al. 2022). Desyibelew & Wondifraw (2019) suggested that these are the most important points to consider when evaluating milk quality and the improvement of milk yield and composition.

The production of sheep milk in Kosovo takes place mainly in mountain areas and is almost exclusively used as a raw material for dairy products. Production of sheep milk in Kosovo is low due to the small number of sheep, which according to the Kosovo Agency of Statistics, (2021) is 211,354. In addition, the seasonal nature of milk production, environmental conditions, and management systems present significant difficulties. Among the most represented sheep breeds in Kosovo is the crossbred 'Sharri' sheep (Fig. 1),which is reared according to an ancient tradition in the mountains of 'Sharri', from where it gets its name. The meadows and pastures of this area are the very basis of this sheep rearing enterprise which is a very important economic potential of Kosovo. In terms of body size, crossbred 'Sharri' sheep can be classified in the group of smallest sheep in Kosovo. The mean height at withers for mature animals is about 62 cm (Bytyqi & Mehmeti, 2006). Sheep are kept for triple purpose (milk-meat-wool), whereas milk production is about 50–90 litres per lactation.

Figure 1. Crossbreed 'Sharri' sheep.

Influence of altitude in milk composition and properties. The influence of altitude in milk composition and properties has been studied by many authors. For example, Mohammed et al. (2022) studied the effects of altitude (14 and 2,110 m above sea level) on some quality parameters of sheep milk and concluded that high altitude affects the increase in pH, total solids, ash, density and calcium, and the decrease in conductivity, moisture, and potassium content. On the other hand, Kohler et al. (2013) found that altitude (2,000 to 2,600 m and 400 m) had no effect on milk production and vitamin D concentration in milk from East Friesian sheep. The same conclusion was offered by Correddu et al. (2021) for the fatty acid profile of Sarda sheep milk at three different altitudes, highlighting the influence of the seasonality of the botanical composition of grazed pasture. Cabiddu et al. (2022) stated that dairy sheep grazing management and pasture botanical composition affect some milk components such as lactose, milk fatty acid profile, phenol content etc.

The influence of lactation period in milk composition and properties. There are also numerous research articles on the influence of lactation period in composition and properties of milk. For instance, Aganga et al. (2002) found that protein, fat, and lactose content in Tswana sheep milk increases during lactation. Bielińska-Nowak' & Czyżak-Runowska, (2016) studied the influence of the lactation period in the milk of East Friesian sheep and concluded that at the end of lactation there was a significant increase in the concentration of total solids, protein, and fat with a simultaneous decrease in lactose concentration compared to the peak of lactation. Bencini (2001) studied the milk composition of Sarda, Awassi, and Merino sheep and found that the concentrations of fat, protein, total solids, and somatic cells are high at the beginning and end of lactation and low at the peak of lactation, while lactose concentration follows closely the lactation yield. Moreover, Michlova et al. (2014) studied the influence of lactation period on vitamin A and E content in raw sheep milk of the East Friesian, Romanov and Lacaune sheep breeds. They stated the lowest content of vitamins was found in early lactation (April) and the highest values appeared at the end of lactation (September). Assan (2015) emphasized that systematic studies on the influence of lactation on milk yield and milk composition are of utmost importance to evaluate the milk production ability of milking animals. There are some studies related to chemical composition (Ramadani et al., 2009a) and physical properties (Ramadani et al., 2013) of individual Sharri sheep milk, and chemical composition of bulk milk (Ramadani et al., 2009b), but there is no study on the effect of altitude.

The objective of this study aimed to compare the changes in chemical composition and physical properties of bulk milk from crossbred 'Sharri' sheep, depending on the altitude (1,200 m - variant A, and 1,600 m - variant B) and lactation period. Considering that the location of sheep farms varies during the grazing season and flocks in these areas move at different altitudes, the importance of this research was to give recommendations for farmers on identifying the best altitude to improve the quality of sheep milk.

MATERIALS AND METHODS

Study area and sheep flocks

According to the objective of the study, raw bulk milk samples were collected from a total of 10 flocks of crossbred 'Sharri' sheep, which were located in two different altitudes of the 'Sharri' Mountains in Kosovo:

- 1,200 m. (variant A, location named Prevallë, latitude: 42° 12' 22.57" N, and longitude: 20° 58' 26.15" E), and
- 1,600 m. (variant B, location named Brod, latitude: 41° 59' 30.84" N, and longitude: 20° 42' 21.96" W).

In this experiment, at each altitude, 5 flocks consisted of over 500 sheep were selected. Sheep mostly belonged to second and third lactation. During the winter season (November to the end of March), the sheep are kept on the farm in the lowlands, where they grazed near the farm. The lambing period is usually seasonal and ended in mid-March. After lambing, the milk is used only for feeding the lambs. According to the tradition of the area, adult lambs, are sold and slaughtered during May. Afterwards, sheep flocks are moved to the highlands, away from urbanization and industrialization. The mountain grazing time started from the end of May to September, where the sheep mainly grazed on natural pasture, but sometimes they were fed with cut grass and supplemental concentrate feeds. The processing of milk into cheese and other milk products began after the weaning period, which occurs about 90 days after lambing (Bytyqi et al., 2014), and included the months of June, July, August, and September.

Considering that the farms included in our experiment were in ecologically clean mountainous areas, in the future they can be treated as opportunities for organic sheep milk production. For this purpose, models of organic livestock farms of Estonia can be taken as options (Lepasalu et al., 2009).

Milk sampling

At altitude of 1,200 m and 1,600 m, 10 samples of raw milk were collected from 10 different sheep flocks during evening and morning milking. The milk was collected in a tank as bulk milk. Bulk milk samples were taken once a month, in triplicate, during June, July, August and September 2021. In total, 120 bulk milk samples were taken during the experiment according to the data presented in the Table 1. Milk samples were collected in sterile bottles according to ISO 707:2008 (IDF 50:2008), (2008), preserved with a sodium azide and transported by hand refrigerator to the laboratory for analyses.

Table 1. The experiment design of milk sampling (*n* = 120)

Altitude	Flocks No.	Samples per flock (One sample per month)	Samples replication	Total samples per flock	Total samples per altitude
A (1,200 m)	1	4	3	12	60
	2	4	3	12	
	3	4	3	12	
	4	4	3	12	
	5	4	3	12	
B (1,600 m)	1	4	3	12	60
	2	4	3	12	
	3	4	3	12	
	4	4	3	12	
	5	4	3	12	
Total samples per experiment					120

Milk analyses

Milk fat, solids non-fat, proteins, lactose, freezing point, and density were analyzed using the Lactostar milk analyzer, model number 3510, Funke Gerber, Germany (using the combined thermo-optical method). The content of total solids was calculated using milk fat and solids non-fat values. The ash content was determined by burning method in furnace oven at 550−600 °C (ISO/CD 9877 |IDF 258). Ash content was calculated by dividing the weight of the ash by the weight of the milk sample as follow:

Ash content (%) = weight ashed sample (g) / weight of milk sample (g) x 100.

The value of titratable acidity was determined by titration, (ISO/TS 11869:2012 |IDF/RM 150:2012). Active acidity (pH) value was measured using pH meter GLP 21 'Crison' (ISO 11869:2012). All measurements were performed in triplicate at the Laboratory of Food Technology in the Faculty of Agriculture and Veterinary in Prishtina, University of Prishtina 'Hasan Prishtina', Kosovo.

Statistical analyses

Collected data on chemical composition and physical properties of raw milk were analysed to find the effect of altitude, lactation period, and their interaction. Statistical analyses were done using JMP IN 7 statistical software (business unit of SAS). Two factor ANOVA is used to find do means differ, and to what extent. The borderline of significance was set to 0.05 and Tukey-Kramer HSD post hoc test was used to compare mean group differences. Effects were significant when $P < 0.05$.

RESULTS AND DISCUSSION

Milk composition and physical properties of crossbred 'Sharri' sheep in different altitudes. Table 2 shows the milk composition and physical properties of crossbred 'Sharri' sheep in two different altitudes. From the results, the chemical composition and physical properties of milk has been good and suitable for consumption or production of cheese and other dairy products, with values within the limits provided by the Administrative Instruction MA−No. 20/2006. Generally, the analysed total milk is characterized by composition and properties as follow: total solids (20.83%), milk fat (8.77%), solids non-fat (12.06%), proteins (5.72%), lactose (5.39%), ash (0.94%), freezing point (-0.66 °C), titratable acidity (12.49 °SH), active acidity (6.71), and density value (1.040 gr cm^{-3}).

Table 2. Crossbred 'Sharri' sheep milk composition and physical properties in two different altitudes (Mean ± SEM) (n = 120)

Parameters	Altitude		Overall	*P* value
	A (1,200 m)	B (1,600 m)		
TS (%)	18.84 ± 0.83^{a}	21.91 ± 0.91^{b}	20.83 ± 0.68	0.0167
MF (%)	7.73 ± 0.36^{a}	9.80 ± 0.40^{b}	8.77 ± 0.31	0.0004
SNF (%)	11.10 ± 0.49	12.11 ± 0.57	12.06 ± 0.38	ns
P (%)	5.30 ± 0.27^{a}	6.14 ± 0.24^{b}	5.72 ± 0.19	0.0243
L (%)	4.95 ± 0.25^{a}	5.83 ± 0.24^{b}	5.39 ± 0.19	0.0159
Ash (%)	0.85 ± 0.04^{a}	1.04 ± 0.06^{b}	0.94 ± 0.04	0.0124
FP (°C)	-0.642 ± 0.01	-0.679 ± 0.01	-0.66 ± 0.01	ns
TA (°SH)	11.84 ± 0.47^{a}	13.13 ± 0.44^{b}	12.49 ± 0.33	0.0492
AA	6.70 ± 0.04	6.72 ± 0.03	6.71 ± 0.02	ns
D (g cm^{-3})	1.040 ± 0.00^{a}	1.049 ± 0.00^{b}	1.040 ± 0.00	0.0408

TS – Total solids (%); MF – Milk fat (%); SNF – Solids non-fat (%); P – Proteins (%); L – Lactose, (%); Ash (%), FP – Freezing point (°C); TA – Titratable acidity (°SH); AA – Active acidity (pH); D – Density (g cm^{-3}); ns – nonsignificant. Means with different superscript in each column ($^{a,\ b}$) differ significantly ($P < 0.05$).

Milk samples from altitude 1,200 m (variant A) contained on average 18.84% total solids, 7.73% milk fat, 11.10% solids non-fat, 5.30% proteins, 4.95% lactose, 0.85% ash, whereas freezing point value vas -0.642 °C, titratable acidity 11.84 °SH, active acidity 6.70, and density value 1.040 g cm^{-3}. Milk samples from altitude 1,600 m - (variant B) contained on average 21.91% total solids, 9.80% milk fat, 12.11% solids non-fat, 6.14% proteins, 5.83% lactose, 1.04% ash, whereas freezing point value was -0.679 °C, titratable acidity 13.13 °SH, active acidity 6.72 and density value 1.049 g cm^{-3}.

The analysed milk in altitude 1,600 m showed a significant increase of 14.01% in total solids, 21.12% in milk fat, 13.68% in proteins, 15.09% in lactose, 18.27% in ash, 9.82% in titratable acidity, and 0.86% in density, whereas changes in solids non-fat, in freezing point, and 0.30% in active acidity have been statistically non-significant.

The effect of altitude in sheep milk quality was an object of research of many authors. Thus, Mohammed et al. (2022) investigated the effect of altitude on some physico-chemical properties of milk and on the concentration of minerals from two altitudes: 14 m and 2,110 m. They found out increase of total solids, ash, density, and active acidity in high altitude, respectively from 25.80% to 26.50%; 0.51% to 4.67%;

1.030 to 1.040 and 6.24 to 6.54, compared to low altitude. Results of our study are relatively lower in total solids content, but the trend of increasing the values are like those of above-mentioned authors. Fotina & Zazharska (2016) have analysed the effects of altitude 251, 309, 341, 376, 394, 524, 580 and 750 m of Zakarpattya valleys on the quality and safety of milk samples. They found decrease of values in altitude 750 m compared to altitude 251 m for fat content in the sheep milk (from 3.83% to 3.27%); solids non-fat (from 11.02% to 10.36%); protein (from 4.14% to 3.82%); lactose (from 6.19% to 5.75%), density (from 1.0397 to 1.0368) and pH value (from 6.66 to 6.51), as well as increase of freezing point from -0.727 °C to -0.681 °C. These results were almost the same for many quality parameters compared to ours. In addition, an opposite trend of decrease in these values is observed. Cividini et al. (2019) studied the milk of indigenous Bovec sheep in different altitude: 480 m, 1,100–1,300 m, 1,600–1,900 m, and 2,100–2,200 m, respectively. They concluded a significant decrease ($P < 0.001$) in the fat content between milk from 480 m (6.1%) and 1,100–1,300 m (5.04%) and a significant increase between milk from 1,600–1,900 and 2,100–2,200 m to 5.6% and 6.78%. Even here, results reported from this study regarding the trend of increasing of fat content are in the same direction for fat content in our study. Merlin et al. (2015) observed chemical and microbiological characteristics of sheep milk produced on two rural properties located in southern Brazil, which were in different locations (Parana–PR, altitude 780 m and Rio Grande do Sul–RS, altitude 690 m). The chemical composition of sheep milk was 17.32% total solids, 5.86% total protein, 7.28% fat, 0.93% ash, and 3.41% lactose. Milk samples from two different dairy sheep properties showed similar chemical composition and physical properties. Significant differences ($P < 0.05$) were observed only in the mean of total solids (PR = 16.47, RS = 18.71%); acidity (PR = 0.22, RS = 0.26% lactic acid), and freezing point (PR = -0.571 °C, RS = -0.583 °C). Results reported from this study regarding the trend of decreasing the total solids content are on the contrary direction, compared to ours. Martini et al. (2008) revealed significant differences ($P < 0.01$) between milk from the lowland and milk from the hill in terms of chemical and hygienic quality. In the hill, the milk had a higher percentage ($P < 0.01$) of total solids (18.29%), protein (5.95%), fat (7.10%), and solids non-fat (11.26%), whereas it has a lower percentage of lactose (4.45%) compared to lowland 16.91%, 5.55%, 5.94%, 10.92%, and 4.51% respectively. Even here, results reported from this study regarding the trend of increasing of almost all parameters in the hill are in the same direction as the results of our study.

Milk composition and physical properties of crossbred 'Sharri' sheep during lactation period. Table 3 shows the milk composition of crossbred 'Sharri' sheep during the four month of lactation period. The quality of the analysed milk was within the norms described in the Administrative Instruction MA–No. 20/2006. The analysed milk during the four month of lactation period showed a significant increase ($P < 0.05$) in total solids (from 15.65 to 25.46%); milk fat (from 6.49% to 10.80%); solids non-fat (from 9.16% to 14.66%); proteins (from 4.46% to 6.94%); lactose (from 3.96% to 6.52%); ash (0.74% to 1.20%); titratable acidity (9.90 to 14.53 °SH); while changes in freezing point (-0.611 to -0.709 °C), active acidity (6.68 to 6.76), and density (1.035 to 1.053 gr cm^{-3}) were statistically non-significant. Like our results, a statistically significant influence of the stage of lactation ($P < 0.001$) is also reported in the research of Tatar et al. (2022), who confirmed the same trend of increasing total solids, total proteins, and fat content.

The value of total solids content during the lactation period increased significantly by 38.53% ($P < 0.0001$). This increase can be explained by the possible influence of environmental factors such as pasture grazing, which should be considered in future studies. Many authors have researched the effects of lactation on sheep milk composition and total solids content. For instance, Tatar et al. (2022) determined changes in total solids content during the lactation period of Lacaune sheep (from 14.80% to 27.73%) with a difference of 46.63%, but this difference is higher than in our research (38.53%). Also, Kuchtík et al. (2008) reported the significant increase of the contents of total solids (from 15.59% to 20.68%) obtained by a total of 27 sheep of the East Friesland breed. Jaramillo et al. (2008) stated the lowest content of total solids in milk, observed in early lactation (18.6%), followed by a moderate increase in mid lactation (19.0%), reaching a markedly higher value at the end of lactation (19.8%). Prpić et al. (2003) and Pavić et al. (2002) in their research on the quality of sheep milk have found the mean value of total solids 19.04%, and 19.11% respectively. The last - mentioned authors reported significantly higher ($P < 0.01$) content of total solids in late lactation compared to the beginning. Moreover, Ochoa-Cordero et al. (2002) stated significant effect of lactation week (weeks 1, 3, 6, 8, and 12), in the milk from 45 Rambouillet sheep's, with the mean value for total solids 16.7 ± 2.1%, which was lower, compared to our results. The lower mean value compared to our results was found by Aganga et al. (2002) in Tswana sheep milk. They reported the effect of lactation period on the nutrients and concluded higher values of total solids (21.34%) at 166 days' post–partum, compared to 15.02% at 40 days' post-partum.

Table 3. Changes of crossbred 'Sharri' sheep milk composition and physical properties during lactation period (Mean ± SEM) (n = 120)

Parameters	Month of lactation period				Difference (June – September) (%)	P value
	June	July	August	September		
TS (%)	15.65 ± 0.57^{d}	19.83 ± 0.78^{c}	22.37 ± 0.08^{b}	25.46 ± 0.75^{a}	38.53	< 0.0001
MF (%)	6.49 ± 0.33^{c}	8.44 ± 0.41^{b}	9.34 ± 0.46^{b}	10.80 ± 0.35^{a}	39.91	< 0.0001
SNF (%)	9.16 ± 0.27^{c}	10.89 ± 0.33^{b}	11.72 ± 0.55^{b}	14.66 ± 0.50^{a}	37.52	< 0.0001
P (%)	4.46 ± 0.18^{d}	5.38 ± 0.20^{c}	6.11 ± 0.26^{b}	6.94 ± 0.33^{a}	35.73	< 0.0001
L (%)	3.96 ± 0.15^{c}	5.18 ± 0.25^{b}	5.90 ± 0.28^{a}	6.52 ± 0.18^{a}	39.26	< 0.0001
Ash (%)	0.74 ± 0.03^{c}	0.83 ± 0.05^{c}	1.01 ± 0.04^{b}	1.20 ± 0.08^{a}	38.33	< 0.0001
FP (°C)	-0.611 ± 0.02^{a}	-0.645 ± 0.02^{ab}	-0.676 ± 0.02^{bc}	-0.709 ± 0.02^{c}	13.82	ns
TA (°SH)	9.90 ± 0.30^{d}	12.14 ± 0.30^{c}	13.38 ± 0.28^{b}	14.53 ± 0.57^{a}	31.87	< 0.0001
AA	6.68 ± 0.03	6.73 ± 0.07	6.68 ± 0.03	6.76 ± 0.05	1.18	ns
D (g cm^{-3})	1.035 ± 0.00^{b}	1.046 ± 0.00^{ab}	1.043 ± 0.00^{ab}	1.053 ± 0.00^{a}	1.71	ns

Note: TS – Total solids (%), MF – Milk fat (%); SNF – Solids non-fat (%); P – Proteins (%); L – Lactose (%); Ash (%); FP – Freezing point (°C); TA – Titratable acidity (°SH); AA – Active acidity (pH); D – Density (g cm^{-3}); ns – nonsignificant. Means with different superscript in each column ($^{a, b, c, d}$) differ significantly ($P < 0.05$).

Milk fat is one of the most variable parameters of milk. In this study, as the lactation period progressed, this quality parameter increased significantly by 39.91% ($P < 0.0001$). The increase in milk fat content may be explained because of the botanical composition of the pasture (milk produced during summer has a higher moisture content than that produced in autumn, and thus a lower fat content). Almost similar results for fat content were obtained by Kawęcka et al. (2020) in the milk of three mountain sheep breeds: Podhale Zackel, Polish Mountain sheep, and Coloured Mountain sheep. Fat content increased to 10.23% in September compared to 6.3% in May. Oravcová et al. (2007) in the milk of three different sheep (Tsigai, improved Valachia and Lacaune) reported high variation in fat content throughout the lactation period: 7.77% for Tsigai, 7.48% for improved Valachian and 6.97% for Lacaune. Our results show an easier increase in fat content compared to those of Tatar et al. (2022) (4.23–14.75%), but in both cases the stage of lactation has shown a statistically significant influence ($P < 0.0001$ and $P < 0.001$). Lower fat content in comparison to value from our study is reported by Pavić et al. (2002) in 202 samples of sheep milk, who obtained the mean 7.52%, which was significantly higher ($P < 0.01$) in the middle and at the end of the lactation period compared to the beginning. Vršková et al. (2015) observed an increase in the fat content of Tsigai sheep milk in value of 8.86% in June compared to 6.99% in April. An increase of all analysed milk parameters during lactation is reported by Komprej et al. (2012) in sheep of Bovec, Improved Bovec, and Istrian Pramenka breeds. Overall means for fat content were 6.59%, 6.22%, and 7.20% respectively, which were also lower, compared with the results reported in our study. According to Konečná et al. (2019), milk fat increased significantly ($P < 0.05$), from 7.1% (day 62) to 7.89% (day 195) in Lacauna sheep milk. A similar statement about the significant effect of lactation period on milk composition and milk fat values of 4.96–7.80% is reported by Kuchtík et al. (2008) in 27 sheep of East Friesland breed.

Solids non-fat content according to Administrative Instruction MA 20/2006, should not be lower than 9.5%. The results for the mean value of this parameter in our study were slightly lower only in June (9.16%), while in the following months there was a significant increase by 37.52% ($P < 0.0001$) until the last month of the lactation period (14.46%). Prpić et al. (2003) found that the mean value of solids non-fat in sheep milk was 11.06%, which was lower than the results of the present study. A highly significant effect of lactation period on the solids non-fat content of milk from sheep of two Greek breeds, with an average value for Karagouniki (11.53%) and Serron (11.10%), is reported by Polychroniadou & Vafopoulou (1985). In addition, Kuchtík et al. (2008) observed an increase in solids non-fat content during lactation (from 10.64% to 12.88%) in 27 sheep of the East Friesian breed reared in a small sheep farm in Juřinka in the Wallachian region.

Protein content significantly increased by 35.73% ($P < 0.0001$) during the lactation period in this study. The mean value of total protein in our research (5.72%) is almost similar (5.74%) as those reported by Tatar et al. (2022). An increase in protein content during lactation period is reported by Komprej et al. (2012) in three dairy sheep breeds (Bovec, Improved Bovec, and Istrian Pramenka) with overall mean values of 5.53%, 5.33% and 5.63%, respectively. Genkovski (2006), found a progressive trend of proteins in the sheep breeds Karakachanska, Srednostaroplaninska and Tetevenska in the four-month examination period, from 5.22–5.45% in April to 6.26–6.58% in July. Konečná et al. (2019) concluded in the milk of eight sheep between May and September that the period of lactation influences the protein content, which ranges from 4.92% to 6.6%. A similar conclusion for protein content was reported by Manfredini et al. (1993), with significantly lower values observed at the beginning of the lactation period than at the end (5.38 and 7.11%). Significantly higher ($P < 0.01$) protein content at the middle and end of the lactation period is confirmed by Pavić et al. (2002) in 202 sheep milk samples with a mean value of 5.90%. Çelik et al. (2003) also found that protein content of Awassi sheep milk was 5.19% and significantly ($P < 0.01$) dependent on lactation period.

Lactose content in this study during the lactation period increased significantly by 39.26% ($P < 0.0001$). Significant effect of lactation period on lactose content emphasized Jaramillo et al. (2008) in organic milk from two Spanish sheep breeds (Guirra & Manchega), and Kraličkova et al. (2012) in sheep milk of East Friesian breed. Ochoa-Cordero et al. (2002) reached the same conclusion in the milk of Rambouillet sheep with a mean value of 4.5 ± 0.4%. On the other hand, Manfredini et al. (1993) reported opposite trends and concluded that sheep milk contains significantly higher lactose content at the beginning of the lactation period than at the end (4.97% and 4.09%). Morand-Fehr et al. (2007) emphasized the same conclusion, who found that lactose content decreases during the lactation period. Moreover, the highest lactose content at the beginning (4.97%) and the lowest at the end (4.09%) of the lactation period is found by Talevski et al. (2009) in sheep milk as a raw material in the dairy industry of Macedonia. On the other hand, Konečná et al. (2019) emphasized that the lactose content in sheep milk was relatively stable during the lactation period and no significance was found.

Ash content in this study gradually increased by 38.33%, with statistically significant influence of lactation period ($P < 0.0001$). A slightly lower value of ash (0.91 ± 0.06%) compared to our results is reported by Ochoa-Cordero et al. (2002) in the milk of 45 Rambouillet sheep. They also emphasize that the lactation week has a significant effect on ash content. On the other hand, Williams et al. (2012) recorded an average ash content of 0.76% in the milk of West African Dwarf sheep and stated that the lactation period had no effect on ash content. Mehaia (1996) reported a stable mean ash content of 0.86%, 0.87% and 0.86%, respectively, in sheep milk from Najdi, Australian and Najdi x Australian bred, respectively, in the central region of Saudi Arabia.

The freezing point value of sheep's milk should not be higher than -0.560 °C according to the Administrative Instruction MA 20/2006. The freezing point complied with this regulation and ranged between -0.611 °C and -0.709 °C. During the lactation period, the freezing points decreased slightly by 13.82%, and the differences between months were nonsignificant. In comparison with the results of the current study, Prpić et al. (2003) in their study on the quality of sheep milk for the production of Krčki cheese and Pavić et al. (2002) in their study of 202 sheep milk samples found a relatively higher mean value of freezing point (-0.555 °C), noting a significant influence of lactation ($P < 0.01$) on this parameter (-0.564 to -0.570 °C). In addition, Mayer & Fiechter, (2012) reported a higher freezing point value (-0.544 °C) in their study in Austria compared to the results of this study and emphasized that some samples showed a higher freezing point value, probably because water was added.

Titratable acidity in this study increased significantly by 31.87% ($P < 0.0001$) during the lactation period. Titratable acidity was slightly higher than in the Administrative Instruction MA 20/2006 (max. 12 °SH) during the whole lactation period. Kawęcka et al. (2020) reported a value of titratable acidity from 9.18 °SH in May to 13.04 °SH in September, with an average value of 11.34 °SH in sheep milk from three mountain breeds (Podhale Zackel, Polish Mountain sheep and Colored Mountain sheep). Pavić et al. (2002) found that the average values of titratable acidity of milk at the beginning (10.01 °SH) and in the middle of lactation (9.30 °SH) were significantly higher ($P < 0.01$) than at the end (8.39 °SH). According to Konečná et al. (2019), the lactation period length influenced the titratable acidity with a mean value of 9.96 °SH, which slightly increased from 8.93 to 10.9 °SH at the end of lactation. On the other hand, Sahan et al. (2005) reported that titratable acidity of milk in Awassi sheep decreased ($P < 0.05$) during lactation and reached its lowest values in the last week of lactation, emphasizing the role of protein in these acidity variations. All the above results were lower than the values obtained in the present study.

Active acidity in this study was stable during the lactation period and showed non-significant differences. Moreover, this parameter was within the values prescribed in the Administrative Instruction MA No. 20/2006, which should be between 6.5 and 6.7. The reported results for active acidity are also almost similar to those reported by Bonczar et al. (2003) in the milk of long wool Polish sheep; Prpić et al. (2003) on the quality of sheep milk for the production of Krk cheese with a mean value of active acidity of 6.66 and Assenat (1991) in the milk of Laucane sheep (6.60 to 6.68). In addition, Konečná et al. (2019) concluded that the lactation period had non-significant influence on active acidity with a mean value of 6.59 in eight Lacaune breed sheep from May to September. However, Pavić et al. (2002) reported that the mean active acidity values of milk were significantly lower ($P < 0.01$) at the beginning (6.66) and middle of lactation period (6.81) than at the end (6.89).

The density value in this study increased slightly by 1.71% during the lactation period, although the differences were non-significant. The density value in the current study is higher than that of Assenat (1991), who reported the average density of sheep milk as 1.036 and emphasized that it increases until the middle of the lactation period and decreases until the end of the lactation period, reaching a value of 1.034 g cm^{-3}. A slightly lower value for the density of milk (1.0342 g cm^{-3}) compared to our study is reported by Bonczar et al. (2003) for the milk of long wool Polish sheep and Brito et al. (2006) for the milk of Lacaune sheep from southern Brazil (1.036 g cm^{-3}). On the other hand, Kuchtík et al. (2008) concluded that the density slightly decreased until the 129th day of lactation. However, thereafter the opposite trend was observed, and the highest value was recorded at the end of lactation (1.036 g cm^{-3}).

Effect of factors altitude and lactation period interaction on crossbred Sharri sheep milk content. The results presented in Table 4, 5 and 6 show that the interactions between altitude * lactation period on crossbred 'Sharri' sheep milk composition (total solids, milk fat, solids non-fat, proteins, lactose, and ash) and physical properties (freezing point, titratable acidity, active acidity, and density) were nonsignificant.

Table 4. Effect of factors Altitude and Lactation period interaction on crossbred 'Sharri' sheep milk content of TS, MF, and SNF, (Mean ± SEM)

Altitude	Month of Lactation	TS, %	MF, %	SNF, %
A (1,200 m)	June	14.20 ± 0.25	5.63 ± 0.13	8.57 ± 0.16
	July	17.55 ± 0.19	7.32 ± 0.12	10.22 ± 0.21
	August	20.14 ± 0.95	8.17 ± 0.45	11.97 ± 0.62
	September	23.47 ± 0.69	9.81 ± 0.13	13.65 ± 0.73
B (1,600 m)	June	17.10 ± 0.61	7.35 ± 0.33	9.75 ± 0.36
	July	22.18 ± 0.36	9.56 ± 0.37	12.56 ± 0.18
	August	24.59 ± 0.33	10.52 ± 0.25	14.07 ± 0.13
	September	27.49 ± 0.27	11.78 ± 0.21	15.67 ± 0.15
P Value		ns	ns	ns

TS – Total solids, MF – Milk fat, SNF – Solids non-fat, ns – nonsignificant.

Table 5. Effect of factors Altitude and Lactation period interaction on crossbred 'Sharri' sheep milk content of P, L, and Ash, (Mean±SEM)

Altitude	Month of Lactation	P, %	L, %	Ash, %
A (1,200 m)	June	4.16 ± 0.13	3.71 ± 0.25	0.69 ± 0.02
	July	4.96 ± 0.16	4.55 ± 0.17	0.71 ± 0.03
	August	5.56 ± 0.37	5.44 ± 0.49	0.97 ± 0.06
	September	6.54 ± 0.63	6.09 ± 0.22	1.03 ± 0.08
B (1,600 m)	June	4.76 ± 0.29	4.20 ± 0.06	0.78 ± 0.06
	July	5.80 ± 0.25	5.80 ± 0.23	0.95 ± 0.04
	August	6.66 ± 0.11	6.34 ± 0.09	1.05 ± 0.07
	September	7.35 ± 0.12	6.95 ± 0.13	1.37 ± 0.06
P Value		ns	ns	ns

P – Proteins, L – Lactose, ns – nonsignificant.

Table 6. Effect of factors Altitude and Lactation period interaction on crossbred 'Sharri' sheep milk physical properties (Mean ± SEM)

Altitude	Month of	FP, °C	TA, °SH	AA, pH	D, g cm^{-3}
A (1,200 m)	June	-0.601 ± 0.02	9.14 ± 0.11	6.68 ± 0.05	1.032 ± 0.00
	July	-0.611 ± 0.01	11.62 ± 0.53	6.72 ± 0.13	1.043 ± 0.01
	August	-0.651 ± 0.02	12.86 ± 0.42	6.68 ± 0.05	1.037 ± 0.00
	September	-0.703 ± 0.02	13.73 ± 0.81	6.73 ± 0.04	1.046 ± 0.01
B (1,600 m)	June	-0.621 ± 0.03	10.66 ± 0.19	6.68 ± 0.05	1.037 ± 0.00
	July	-0.679 ± 0.03	12.66 ± 0.34	6.74 ± 0.07	1.048 ± 0.00
	August	-0.701 ± 0.04	13.89 ± 0.22	6.68 ± 0.05	1.050 ± 0.01
	September	-0.715 ± 0.03	15.33 ± 0.68	6.78 ± 0.09	1.059 ± 0.01
P Value		ns	ns	ns	ns

FP – Freezing point, TA – Titratable acidity, AA – Active acidity, D – Density, ns–nonsignificant.

CONCLUSIONS

Sheep milk in altitude 1,600 m (variant B) in all analysed parameters showed significantly higher values ($P < 0.05$), and lower value of freezing point, compared to milk of altitude 1,200 m (variant A), except solids non-fat, freezing point and active acidity, which were statistically non-significant.

The analysed milk during the four months of lactation period increased significantly ($P < 0.0001$), in total solids (from 15.65% to 25.46%, milk fat (6.49% to 10.80%), solids non-fat (9.16 to 14.66%), proteins (4.46% to 6.94%), lactose (3. 96% to 6.52%), ash (0.74% to 1.20%), titratable acidity (9.90 to 14.53 °SH), while changes in freezing point (-0.611 to –0.709 °C), active acidity (6.68 to 6.76), and density (1.035 to 1.053 g cm^{-3}) were statistically non-significant. The interactions between altitude * lactation period on the composition (total solids, milk fat, solids non-fat, proteins, lactose, ash) of milk from crossbred 'Sharri' sheep and physical properties (freezing point, titratable acidity, active acidity, and density) were non-significant. Therefore, it can be concluded that altitude and lactation period have a significant effect on most milk components of crossbred 'Sharri' sheep milk. Based on the results of this research, recommendations for farmers on identifying the best altitude to improve the quality of sheep milk can be offered. In addition, further studies, especially on the influence of altitude and lactation period or the effect of the pastures, are needed to better explain the differences in sheep milk quality and draw more definitive conclusions.

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