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Assessment of Quality and Safety of Farm Level Produced Cheeses from Sheep and Goat Milk

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Abstract: Consumption of sheep's and goat's milk and cheese is currently increasing. The production process of these types of cheese is being carried out by traditional domestic production at farm level. However, knowledge in the field of hygiene, technology and health safety of cheeses are still insufficient. This study aimed to examine the physical and chemical quality and microbiological safety of sheep's and goat's milk and cheeses made from them. The month of milking influenced the content of milk components ($p < 0.001$) in sheep's milk and goat's milk, but no changes in SCC content during the examined period were found ($p > 0.05$). Level of contamination by *Enterobacteriaceae* sp. and coagulase-positive staphylococci was lower than 5 log CFU/mL in sheep's and goat's milk. During the ripening time, the number of lactic acid bacteria significantly raised ($p < 0.001$). Ripening time statistically changed ($p < 0.001$) not just the microbial safety of cheeses but also the color ($p < 0.01$). Under the applicable regulations, the analyzed samples were evaluated as suitable for human consumption.

Keywords: sheep and goat milk; cheeses; food quality; microbial safety; SCC

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1. Introduction

The high nutritive values of ewe's and goat's milk and dairy products has led to a high demand that is increasing daily worldwide [1–5]. Sheep and goat breeding have recently expanded in the Slovak Republic, primarily on private farms with direct cheese production. The high levels of protein, fat, and calcium in casein unit make sheep and goat milk an excellent matrix for cheese production [6–8].

The Valachian breed is the most represented sheep breed in Slovakia (42%), the second most represented breed is Tsigai (38%) [4,5]. Breed of the Valachian sheep is mostly kept in the extensive/semi-extensive production system localized in foothill and mountain areas more than 800 m above the sea level. They are considered as multi-purpose breeds for milk (cheese) production, production of offspring for slaughter as young animals and wool production [9]. The production of sheep milk is seasonal due to the seasonal fertility of the breeds. Ewes lamb mainly from February to April. After weaning the lambs, milking of the sheep starts and it lasts until autumn [10,11]. Goat farming in Slovakia is a specific livestock sector where most goats are concentrated in small-scale breeding of white shorthair goats, mostly for milk production [12]. The standardized milking period of white shorthair goats is 240 days [13]. The traditional production of sheep and goat cheeses in Slovakia consists in processing the milk within 2 h after milking and then adjusting the temperature to 32 °C. Milk for the production of such cheeses does not have to be pasteurized, according to Government regulation 312/2003 [14].

Slovakia is a landlocked central European country (16–23° E, 47–50° N), bordered with five states: Poland, Ukraine, Hungary, Czech Republic, and Austria. The climate of Slovakia can be described as a typical European continental. The average summer temperature is 21 °C, with July and August being the warmest months. Temperature and precipitation are altitude dependent, with annual precipitation ranging from 450 mm in the southern lowlands to over 2000 mm in the northern High Tatras mountains [15].

Physicochemical analysis is an important tool for the examination of the quality of dairy products. Determination of physiochemical properties of milk and dairy products as cheeses is important for assessment of the quality of milk products and examination the concentration of milk components [6].

Food safety is achieved on the one hand by focusing on prevention, following good hygiene guidelines and the principles of hazard analysis and critical control points (HACCP), and on the other hand by meeting the microbiological criteria laid down in Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs [16]. The microbiological quality of traditional home-made cheeses and their safety for consumers depend on the microbiological quality of the raw milk used for production, compliance with environmental hygiene standards, personal hygiene of workers, as well as other possible contamination after processing [17–20]. The most important group of microorganisms in ripened cheeses are lactic acid bacteria (LAB). Lactic acid bacteria are also capable of inhibiting the growth of other bacteria such as those of the *Enterobacteriaceae* (EB) family or coliforms. The presence of coliforms, *E. coli*, and enterococci in sheep's and goat's milk cheese indicates poor adherence to good hygiene practice guidelines during the technological processing [21,22]. Microbial analysis and somatic cell count (SCC) have been used to diagnose subclinical mastitis (SCM) in ewes and goats. This condition can affect milk yield, milk composition, and final quality of dairy products [23]. In Slovakia the Regulations (EC) nos. 852/2004 and 853/2004 European legislation lays down general food hygiene rules and specific ones for food of animal origin. However, the Regulation (EC) no. 853/2004 does not define SCC limits for sheep and goat milk [24].

A number of studies have been carried out around the world on the composition and quality of milk, which are influenced by various factors. There is not enough information from local studies on the nutritional composition of milk produced at farm level. A study on the composition of locally produced milk can provide a wealth of data on nutrient content and can be compared to other parts of the world. To improve its benefits, it is possible to evaluate the quality of milk and products made from it [25].

In the border area we have chosen, where is little infrastructure and high demand for sheep's and goat's milk products, there is little or no awareness of the population in the field of health safety for the consumer. Milk and dairy products are important for family consumption and also as a source of income through the sale of dairy products. The quality and safety of cheeses, especially made on the farm level, are the result of many factors, including the effects of seasonal climate, hygiene during the making process as well as ripening time [26].

The aim of this study was to examine the physical, chemical, and microbiological properties of the Valachian sheep and white shorthair goat milk and cheeses produced on the farm level.

2. Materials and Methods

2.1. Source of Milk and Cheeses Samples

The present study was conducted during the period of April to September 2020, which represents the traditional milking season in Slovakia. Fresh non-pasteurized sheep and goat milk and cheese made from examinate milk were obtained from a farm located in the border area of Slovakia and Hungary in region Slanské vrchy (Figure 1). This region is placed around the volcanic mountains with the same name Slanské vrchy, which stretches north-south direction to the border with Hungary. The farm raises Valachian sheep and white shorthaired goats, which were grazed in one flock in a wild pasture. Sheep and goats spend

all day in pastureland, and they were kept in enclosures at night and during milking. The sheep and goats were milked twice a day (morning and evening). The pre-milking phase consisted of forestripping. Milk was obtained by hand-milking process in accordance with the hygiene conditions. Samples of sheep's and goat's milk were taken from the morning milking. The samples of milk and cheeses made from it were placed in the sterile sample containers and transported at 4 °C without the addition of preservatives, to the laboratory at the University of Veterinary Medicine and Pharmacy in Košice for analyses. Within 4 h of collection, samples of raw milk and cheeses made from it were subjected to physical, chemical and microbiological analysis. A total of 144 samples were collected over the six months period in a ratio 1:1 (sheep:goat). The cheeses were made from un-pasteurised and non-standardized milk without adding a cheese starter culture, in the traditional way of production [27]. All experimental cheeses were sampled in the main production season, which starts in April and ends in October/September. Samples of individual cheeses, where one sample of cheese represented 500 g, were subjected to physical, chemical and microbiological analysis on the 1st, 6th and 12th day of ripening. During ripening, all the cheeses were stored in a ripening chamber at 10–12 °C and 88–89% relative humidity. The research was carried out in accordance with the order of the Government of the Slovak Republic. This study and results have not been affected by Covid 19, whether at the level of primary production, milk processing or analysis of milk and cheese samples.

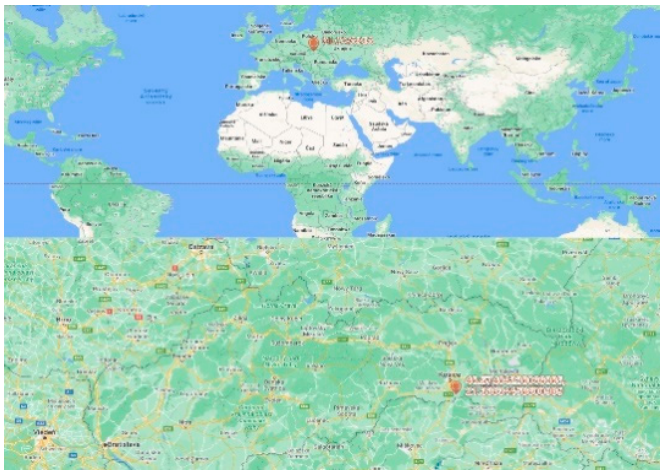

Figure 1. Geographic location (based on equator) of samples collection region (GPS).

2.2. Physical and Chemical Parameters

Selected physical and chemical properties were determined from the examined samples of milk and cheese made from it.

The content of fat, protein, lactose, solids non-fat (SNF), added water (Adw) and milk density of sheep and goat milk samples was determined using a Lactoscan MCCW milk samples analyzer (Milkotronic Ltd., Nova Zagora, Bulgaria) [28] previously calibrated for the analysis of raw sheep and goat milk by the manufacturer using standard methods and procedures. The pH of milk samples was determined with a pH meter 7110 (Wissenschaftlich-Technische Werkstätten, Germany), which was calibrated using standard buffer solutions 4.01 and 7.00 pH (Wissenschaftlich-Technische Werkstätten, Germany) before each determination. The titrable acidity of milk samples was measured by the Soxhlet–Henkel method (°SH.100 mL^{-1}) [29].

Cheese samples were analyzed for: fat, pH, acidity, dry matter and water activity (aw). The fat content of cheese was determined by Gerber's method. The pH of dispersion was measured potentiometrically using a digital inoLab® pH 340i meter (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) [30]. The Soxhlet-Henkel method (°SH.100 mL^{-1}) was used for analyses of acidity detection [31]. Dry matter was detected by international standard methods [32]. Water activity (a_w) of cheeses was detected by

using the LabMASTER-aw (Novasina AG, Lachen, Switzerland) regularly calibrated [33]. Six measurements of physical-chemical parameters were performed for each sample and the obtained results were subsequently statistically evaluated.

2.3. Somatic Cells Analysis

To detect the somatic cells count, 100 µL of each milk sample were taken into a microtube containing lyophilized Sofia Green dye. After repeated mixing of the sample, 8 µL of the sample were subsequently transferred to the surface of LACTOCHIPx4. Somatic cell counts were detected with a low magnification fluorescence microscope with the fast focusing and counting software Lactoscan SCC (Milkotronic Ltd., Nova Zagora, Bulgaria) [34]. Results of somatic count SCC (10^3 mL^{-1}) were transformed to logarithmic form.

2.4. Microbial Analyses

In addition to physicochemical analysis, microbiological analysis was performed on the obtained samples of sheep's and goat's milk and cheeses. Preparation of test samples, initial suspension and decimal dilutions were prepared according to STN EN ISO 6887-5 (2011) [35]. Subsequently, the presence of *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus* sp. was detected in the examined samples of sheep's and goat's milk and cheese according to the relevant ISO standards. Detection of the number of bacteria of the family *Enterobacteriaceae* was performed according to STN EN ISO 21528-1 (2019) [36] using the selective diagnostic medium Violet Red Bile Agar (VRBL; HiMedia, India). After incubation, suspected colonies were tested for glucose fermentation and oxidase reaction (OXItest; Erba-Lachema, Brno, Czech Republic) to confirm bacteria of the *Entrobacteriaceae* family. Tryptone Bile X-glucuronide medium (TBX; Oxoid, Hampshire, UK) selective diagnostic medium was used to detect the number of β-glucuronidase-positive *Escherichia coli* according to the procedure described in STN ISO 16649-2 (2001) [37]. The number of bacteria of the genus *Bacillus cereus* was determined using the procedure described in STN EN ISO 7932 (2004) [38]. The detection and number of *Listeria monocytogenes* was determined according to STN EN ISO 11290-2 (2019) [39]. The number of coagulase-positive staphylococci (CoPS) was also determined in the examined samples according to STN EN ISO 6888-1 (1999) [40] using Baird-Parker selective-diagnostic medium (HiMedia, India). At the same time, the number of lactic acid bacteria (LAB) was determined in milk and cheese samples according to STN ISO 15214 (2002) [41]. Microbiological values were transported into logarithmic transformation ($\log_{10}$) to approximate the data to a normal frequency distribution in order to correctly apply statistical testing methods (one-way analysis of variance) [42].

2.5. Color Analysis

The color of analyzed cheeses samples was quantitatively measured by a Minolta Chroma meter CR-410 (Minolta, Osaka, Japan) [43] by using International Commission on Illumination values. Color Data SoftwareCM-S100w SpectraMagic NX (Konica Minolta Sensing Inc.) and Chroma meter CR-410 were used for the measurement of colorimetric data. Three color parameters were determined for all samples, L* (lightness), a* (green-red) value, and b* (blue-yellow) value. Color measurements were determined according to the CIELab colorspace system (Commission Internationale de l'Eclairage, 1986) [44]. The cheese samples were analyzed on the 1st, 6th, and 12th day of ripening.

2.6. Statistical Analysis

Numerical data presented in this study are expressed as the mean value for each parameter ± standard deviation. The statistical analysis was performed by one-way analysis of variance. ANOVA and Tukey test for multiple comparison of means with a confidence interval set at 95% was conducted with statistics software GraphPad Prism 8.3.0.538 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Milk Quality and Safety

3.1.1. Physical and Chemical Indicators

The season of production significantly affected all quality traits of milk. The mean value and standard deviation of the selected physical and chemical parameters of sheep and goat milk are summarized in Tables 1 and 2.

Table 1. Means and their standard deviations (SD) of the physical and chemical indicators of sheep's milk depending on the month of milking.

	April	May	June	July	August	September	*p* Value
Fat (%)	5.63 ± 0.92 ^{c}	5.94 ± 0.98 ^{c}	7.81 ± 0.60 ^{b}	8.62 ± 0.96 ^{a}	8.77 ± 0.77 ^{a}	9.01 ± 0.72 ^{a}	<0.001
Protein (%)	5.23 ± 0.56 ^{e}	5.67 ± 0.26 ^{d}	5.97 ± 0.94 ^{cd}	6.27 ± 0.63 ^{c}	6.98 ± 0.44 ^{b}	7.46 ± 0.29 ^{a}	<0.001
Lactose (%)	4.96 ± 0.79 ^{a}	4.57 ± 0.30 ^{b}	4.44 ± 0.35 ^{bc}	4.15 ± 0.47 ^{cd}	4.01 ± 0.41 ^{d}	3.92 ± 0.54 ^{d}	<0.001
SNF (%)	8.88 ± 0.37 ^{f}	9.50 ± 0.29 ^{e}	10.99 ± 0.49 ^{d}	12.28 ± 0.34 ^{c}	12.76 ± 0.39 ^{b}	13.21 ± 0.38 ^{a}	<0.001
Density (g/L)	1033.37 ± 0.33 ^{c}	1033.17 ± 0.27 ^{d}	1033.49 ± 0.17 ^{c}	1034.38 ± 0.28 ^{ab}	1034.26 ± 0.22 ^{b}	1034.48 ± 0.39 ^{a}	<0.001
Ad_w	0.00	0.00	0.00	0.00	0.00	0.00	>0.05
Acidity (°SH)	9.66 ± 0.33 ^{b}	9.74 ± 0.36 ^{b}	10.33 ± 0.34 ^{a}	10.42 ± 0.30 ^{a}	10.45 ± 0.33 ^{a}	9.88 ± 0.43 ^{b}	<0.001
pH	6.65 ± 0.34	6.65 ± 0.38	6.67 ± 0.42	6.71 ± 0.64	6.74 ± 0.59	6.72 ± 0.46	>0.05

$^{a, b, c, d, e, f}$—Means within a row different superscript differ ($p < 0.05$).

Table 2. Means and their standard deviations (SD) of the physical and chemical indicators of goat's milk depending on the month of milking.

	April	May	June	July	August	September	*p* Value
Fat (%)	3.82 ± 0.61 ^{a}	3.37 ± 0.54 ^{b}	3.32 ± 0.58 ^{b}	3.60 ± 0.85 ^{bc}	3.99 ± 0.58 ^{a}	4.15 ± 0.73 ^{a}	<0.001
Protein (%)	3.66 ± 0.49 ^{ab}	3.52 ± 0.55 ^{b}	3.41 ± 0.63 ^{b}	3.26 ± 0.65 ^{b}	3.54 ± 0.71 ^{b}	4.01 ± 0.67 ^{a}	<0.001
Lactose (%)	4.36 ± 0.44 ^{a}	4.22 ± 0.36 ^{ab}	4.17 ± 0.43 ^{ab}	4.17 ± 0.53 ^{ab}	4.01 ± 0.65 ^{b}	3.95 ± 0.52 ^{b}	<0.05
SNF (%)	9.41 ± 0.35 ^{d}	9.50 ± 0.29 ^{d}	9.74 ± 0.39 ^{cd}	9.93 ± 0.72 ^{c}	10.35 ± 0.51 ^{b}	11.10 ± 0.74 ^{a}	<0.001
Density (g/L)	1026.97 ± 0.26 ^{c}	1027.49 ± 0.66 ^{d}	1027.18 ± 0.20 ^{cb}	1028.66 ± 0.34 ^{a}	1028.52 ± 0.41 ^{b}	1027.59 ± 0.34 ^{d}	<0.001
Ad_w	0.00	0.00	0.00	0.00	0.00	0.00	>0.05
Acidity (°SH)	5.81 ± 0.41 ^{e}	4.79 ± 0.54 ^{d}	5.06 ± 0.74 ^{cd}	5.38 ± 0.56 ^{c}	6.27 ± 0.50 ^{b}	6.74 ± 0.46 ^{a}	<0.001
pH	6.83 ± 0.70	6.73 ± 0.58	6.82 ± 0.84 ^{a}	6.79 ± 0.73	6.70 ± 0.54	6.67 ± 0.90	>0.05

$^{a, b, c, d, e}$—Means within a row different superscript differ ($p < 0.05$).

In sheep milk, the month of milking significantly affected all quality traits of analyzed milk (Table 1). The content of fat and solids non-fat increased with the time of lactation ($p < 0.001$). The highest increase was recorded for fat and solids non-fat content, when fat content increased from April 5.63 ± 0.92% to 9.01 ± 0.72% in September and also solid non-fat increased to 13.21 ± 0.38% in September compared to 8.88 ± 0.37% in April (Table 1). Protein concentration in sheep milk, as well as fat and SNF, rose during the lactation period ($p < 0.001$). However, the content of lactose was the lowest at the end of lactation in September ($p < 0.001$). Density of sheep milk was the lowest in May 1033.17 ± 0.27 g/L and the highest in September 1034.48 ± 0.39 g/L. The increasing of density and decreasing of lactose in our study is also related to high somatic cell count. The presence of added water was not detected in any of sheep milk samples. The months of milking affected acidity of sheep milk ($p < 0.001$). The results of detection of pH through the whole period did not showed any significant change ($p > 0.05$).

Chemical composition and physical characteristics of goat milk analyses (Table 2) show significant changes in fat content, especially between June and September ($p < 0.001$). For the fat indicator, there was a relation between the quantity of fat with individual months of milking. Milk in the last month and in the first month of lactation had a higher

protein content than that milk in the mid lactation ($p < 0.05$). A similar trend was found out also for fat content, with significantly higher values in the early and late stages of lactation compared to mid stages of lactation. Protein content was in range of 3.26 ± 0.65% to 4.01 ± 0.67% ($p < 0.001$) (Table 2). Results showed the decreasing of lactose during the month of milking ($p < 0.05$). The highest level was at the beginning of lactation on April 4.36 ± 0.44% and the lowest at the end of lactation period 3.95 ± 0.52%. Non-fat solids and density of goat milk significantly rose during the period ($p < 0.001$). The higher concentration of SNF was examined in September (Table 2). The density of goat milk was unstable during the whole period of time ($p < 0.001$). As well as in sheep milk, no added water was found in goat's milk. This means that no attempt was made to adulterate the milk. Goat milk acidity changed throughout the year ($p < 0.001$). The highest acidity of goat milk was detected in September and the lowest value was detected during May. In accordance, because the higher the acidity, the lower the pH, the pH was higher during the spring and lower during autumn. In April, the pH was 6.83 ± 0.70 compared to September 6.67 ± 0.90.

3.1.2. Somatic Cells Analysis

Somatic cell count is widely used for evaluating milk quality. During the period of study, from April to September, the mean value of SCC in sheep and goat milk has not changed significantly ($p > 0.05$). The highest number of SCC in sheep milk was detected on July 5.79 ± 0.53 $\log_{10}$ SCC and the lowest number of SCC was detected in April 5.51 ± 0.52 $\log_{10}$ SCC.

In goat milk the highest SCC was determinate in September 4.59 ± 0.95 $\log_{10}$ SCC and the lowest 4.31 ± 0.33 $\log_{10}$ SCC in April (Table 3). Mean value of somatic cell count corresponds to the increased prevalence of mastitis during the summer months. The mean value of SCC during the research period was in sheep milk 5.68 ± 0.82 $\log_{10}$ SCC and 4.44 ± 0.67 $\log_{10}$ SCC in goat milk.

Table 3. Means and their standard deviations (mean ± SD) of $\log_{10}$ SCC in sheep and goat milk samples on different months of milking.

	April	May	June	July	August	September	*p* Value
Sheep's milk	5.52 ± 0.61	5.58 ± 0.92	5.67 ± 0.84	5.79 ± 0.86	5.78 ± 1.12	5.76 ± 0.77	>0.05
Goat's milk	4.31 ± 0.33	4.39 ± 0.49	4.41 ± 0.85	4.48 ± 0.63	4.48 ± 0.78	4.59 ± 0.95	>0.05

The month of milking in both cases (sheep milk and goat milk) influenced the content of most milk components, but no changes in SCC ($p > 0.05$) during season research were found. Total solids in milk were found to be lower in goat milk with high SCC. It is well accepted that an increase of SCC causes a decrease in the concentration of lactose in both sheep milk and goat milk. In this study, the highest SCC was determined in August and September, when the prevalence of intramammary gland infection (IMI) is higher. The results show that the highest content of total proteins was recorded in the month with a higher SCC. This condition may be due to the lactation phase. In general, the protein content is high at the beginning of the lactation period, then decreases in the middle of the lactation period, and then increases again at the end of the lactation period. Results showed that the lowest value of somatic cells in goat milk was detected at the beginning of the study period 4.31 ± 0.33 $\log_{10}$ SCC and the highest number of SCC was measured on September 4.59 ± 0.95 $\log_{10}$ SCC, what can be attributed to the fact that in the summer months there is an increase in the prevalence of IMI, while in general the highest occurrence of diseases is recorded in August and September.

3.1.3. Microbial Analyses

As reported in Figures 2 and 3, over the 6 months examined period, the number of bacteria from *Enterobacteriaceae* family ranged from 3.95 ± 0.35 log CFU/mL to

7.08 ± 0.15 log CFU/mL in sheep's milk and 3.49 ± 0.13 log CFU/mL to 7.32 ± 0.19 CFU/mL goat's milk. The results showed significant change ($p < 0.001$) of number of bacteria from *Enterobacteriaceae* family, which increased from April to September.

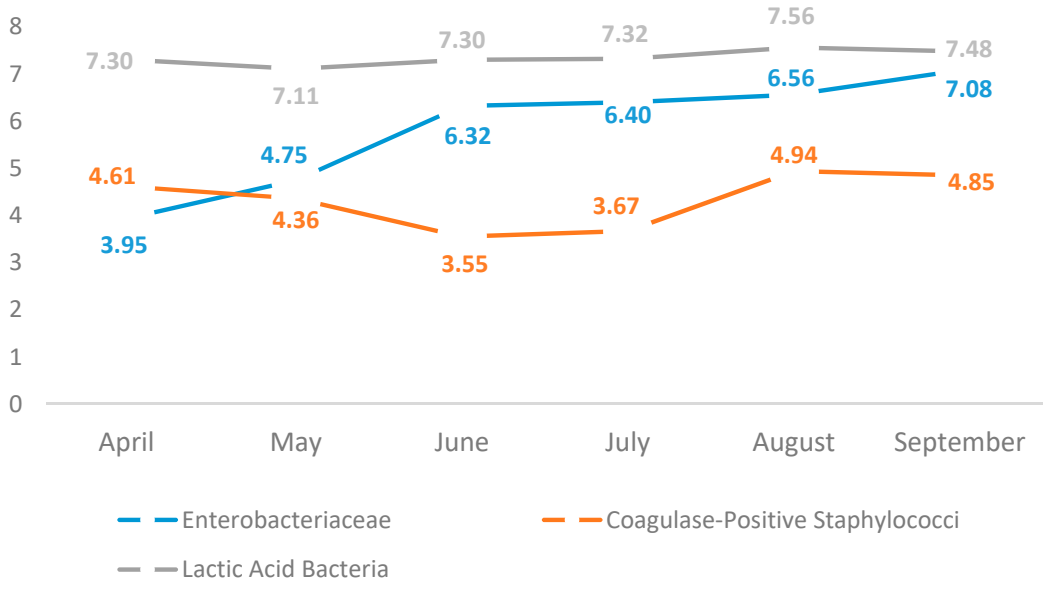

Figure 2. Means and their standard deviations (mean ± SD) of selected bacteria (log CFU/mL) in samples of raw sheep milk.

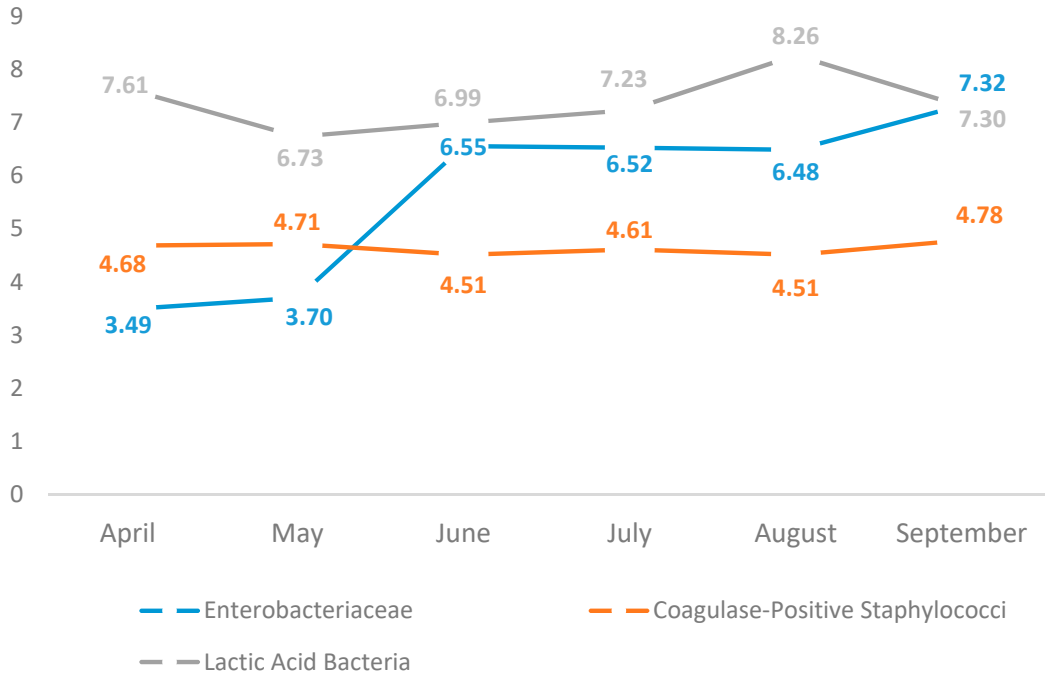

Figure 3. Means and their standard deviations (mean ± SD) of selected bacteria (log CFU/mL) in samples of raw goat milk.

In sheep's milk the mean value of coagulase-positive staphylococci was 4.33 ± 0.57 log CFU/mL with the highest value 4.94 ± 0.15 log CFU/mL in August and lowest 3.55 ± 0.23 log CFU/mL in June. The mean value of coagulase-positive staphylococci in goat's milk was 4.30 ± 0.56 log CFU/mL with the highest value 4.78 ± 0.11 log CFU/mL and the lowest value 4.51 ± 0.24 log CFU/mL. The number of coagulase-positive staphylococci varied significantly ($p < 0.001$) over the course of months in both sheep's and goat's milk. The increase of coagulase-positive staphylococci and

bacteria from *Enterobacteriaceae* family was detected especially in milk samples with higher SCC in our study.

The presence of *B. cereus*, *E. coli*, and *L. monocytogenes* was not detected in any of the analyzed sheep and goat raw milk samples.

Lactic acid bacteria significantly changed ($p < 0.001$) during the examined period. The results showed that the mean value of lactic acid bacteria in sheep's milk was 7.34 ± 0.19 log CFU/mL and in goat milk 7.35 ± 0.50 log CFU/mL. In sheep's milk, the number of lactic acid bacteria increased from May 7.11 ± 0.14 log CFU/mL to 7.56 ± 0.12 log CFU/mL in August (Figure 2). In goat milk the highest number of lactic acid bacteria was 8.26 ± 0.18 log CFU/mL in August and the lowest 6.73 ± 0.06 log CFU/mL in May. In goat's milk samples, the value of isolated lactic acid bacteria fluctuated during the individual months, as indicated in Figure 3.

3.2. Cheeses Quality and Safety

3.2.1. Physical and Chemical Indicators

All physical and chemical indicators examined in sheep cheese and goat cheese samples are reported in Tables 4 and 5. All measurements did not differ much between the sheep and goat cheese samples. In the sheep cheese samples, the fat contents increased during the monitored period (Table 4), as well as in goat cheese samples (Table 5). Fat content was higher in goat cheese samples compared to sheep cheese samples. Detected values of pH in sheep and goat cheese samples decreased, while acidity increased from April to September (Tables 4 and 5). Examined physical and chemical indicators: fat content, pH value, acidity, and dry matter showed significant changes over the months from April to September ($p < 0.001$). However, significant change of water activity was not detected ($p > 0.05$).

Table 4. Means and their standard deviations (mean ± SD) of the physical and chemical indicators of sheep cheese samples at 1st, 6th, and 12th day of ripening.

		April	May	June	July	August	September	*p* Value
Fat (%)	1st day	19.36 ± 0.03 ^{e}	19.44 ± 0.02 ^{d}	19.42 ± 0.01 ^{d}	19.54 ± 0.03 ^{c}	20.77 ± 0.04 ^{b}	23.48 ± 0.03 ^{a}	<0.001
	6th day	20.02 ± 0.05 ^{c}	20.39 ± 0.02 ^{d}	20.02 ± 0.04 ^{d}	20.45 ± 0.01 ^{c}	20.43 ± 0.05 ^{b}	26.44 ± 0.04 ^{a}	<0.001
	12th day	21.35 ± 0.02 ^{f}	21.47 ± 0.07 ^{e}	23.74 ± 0.01 ^{d}	24.56 ± 0.02 ^{c}	25.02 ± 0.04 ^{b}	30.02 ± 0.04 ^{a}	<0.001
pH	1st day	6.52 ± 0.01 ^{a}	6.52 ± 0.03 ^{a}	6.51 ± 0.02 ^{a}	6.49 ± 0.03 ^{b}	5.28 ± 0.01 ^{d}	5.41 ± 0.01 ^{c}	<0.001
	6th day	6.06 ± 0.03 ^{a}	5.57 ± 0.01 ^{b}	5.55 ± 0.03 ^{b}	5.37 ± 0.02 ^{c}	4.96 ± 0.02 ^{e}	5.16 ± 0.03 ^{d}	<0.001
	12th day	5.20 ± 0.02 ^{b}	5.28 ± 0.01 ^{a}	5.26 ± 0.02 ^{a}	5.29 ± 0.02 ^{a}	4.54 ± 0.03 ^{d}	5.02 ± 0.09 ^{c}	<0.001
Acidity (°SH)	1st day	41.32 ± 0.03 ^{f}	48.64 ± 0.02 ^{c}	53.55 ± 0.04 ^{b}	53.57 ± 0.05 ^{b}	68.07 ± 0.02 ^{a}	44.11 ± 0.02 ^{d}	<0.001
	6th day	47.80 ± 0.03 ^{f}	54.02 ± 0.04 ^{e}	65.62 ± 0.02 ^{d}	66.00 ± 0.03 ^{c}	69.12 ± 0.04 ^{b}	71.18 ± 0.03 ^{a}	<0.001
	12th day	76.11 ± 0.03 ^{d}	72.90 ± 0.02 ^{e}	76.08 ± 0.04 ^{d}	78.54 ± 0.02 ^{c}	82.72 ± 0.05 ^{b}	84.36 ± 0.07 ^{a}	<0.001
Dry matter (%)	1st day	44.57 ± 0.03 ^{e}	44.55 ± 0.01 ^{e}	45.09 ± 0.03 ^{d}	46.29 ± 0.07 ^{c}	49.21 ± 0.02 ^{a}	46.39 ± 0.02 ^{b}	<0.001
	6th day	45.61 ± 0.02 ^{d}	49.36 ± 0.03 ^{a}	47.69 ± 0.03 ^{c}	48.15 ± 0.07 ^{b}	49.35 ± 0.03 ^{a}	44.83 ± 0.04 ^{e}	<0.001
	12th day	46.30 ± 0.02 ^{f}	50.89 ± 0.04 ^{a}	49.55 ± 0.02 ^{d}	49.88 ± 0.04 ^{c}	50.15 ± 0.04 ^{b}	47.41 ± 0.02 ^{e}	<0.001
a_w	1st day	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.93± 0.01	0.93 ± 0.01	>0.05
	6th day	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.92 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	>0.05
	12th day	0.92 ± 0.01	0.93 ± 0.01	0.92 ± 0.01	0.92 ± 0.01	0.93 ± 0.01	0.92 ± 0.01	>0.05

$^{a, b, c, d, e, f}$—Means within a row different superscript differ ($p < 0.05$).

Table 5. Means and their standard deviations (mean ± SD) of the physical and chemical indicators of goat cheese samples at 1st, 6th, and 12th day of ripening.

		April	May	June	July	August	September	*p* Value
Fat (%)	1st day	22.44 ± 0.01 ^{e}	22.35 ± 0.02 ^{e}	23.44 ± 0.01 ^{d}	25.16 ± 0.03 ^{c}	29.40 ± 0.02 ^{b}	30.01 ± 0.04 ^{a}	<0.001
	6th day	24.51 ± 0.03 ^{f}	23.51 ± 0.02 ^{e}	25.74 ± 0.02 ^{d}	27.67 ± 0.04 ^{c}	30.02 ± 0.05 ^{b}	32.02 ± 0.05 ^{a}	<0.001
	12th day	25.01 ± 0.04 ^{f}	25.52 ± 0.03 ^{e}	28.15 ± 0.02 ^{d}	30.46 ± 0.03 ^{c}	32.43 ± 0.03 ^{b}	34.63 ± 0.02 ^{a}	<0.001
pH	1st day	6.62 ± 0.03 ^{a}	6.61 ± 0.03 ^{a}	6.56 ± 0.02 ^{b}	5.47 ± 0.03 ^{c}	5.38 ± 0.03 ^{d}	5.06 ± 0.04 ^{f}	<0.001
	6th day	6.07 ± 0.02 ^{a}	5.50 ± 0.02 ^{b}	5.32 ± 0.02 ^{c}	5.32 ± 0.02 ^{c}	5.33 ± 0.03 ^{c}	4.93 ± 0.06 ^{d}	<0.001
	12th day	5.33 ± 0.04 ^{a}	5.10 ± 0.06 ^{b}	5.12 ± 0.01 ^{b}	4.91 ± 0.04 ^{c}	5.00 ± 0.01 ^{d}	4.85 ± 0.01 ^{f}	<0.001
Acidity (°SH)	1st day	42.21 ± 0.05 ^{f}	48.81 ± 0.01 ^{d}	56.40 ± 0.02 ^{c}	56.67 ± 0.03 ^{c}	60.22 ± 0.04 ^{b}	72.11 ± 0.01 ^{a}	<0.001
	6th day	44.18 ± 0.02 ^{f}	52.55 ± 0.03 ^{e}	61.42 ± 0.01 ^{d}	66.72 ± 0.04 ^{c}	78.41 ± 0.01 ^{b}	79.52 ± 0.03 ^{a}	<0.001
	12th day	68.80 ± 0.05 ^{f}	69.21 ± 0.03 ^{e}	71.26 ± 0.05 ^{d}	72.33 ± 0.03 ^{c}	80.60 ± 0.04 ^{b}	82.00 ± 0.03 ^{a}	<0.001
Dry matter (%)	1st day	44.38 ± 0.02 ^{e}	44.36 ± 0.03 ^{f}	45.23 ± 0.01 ^{d}	47.60 ± 0.02 ^{c}	50.89 ± 0.02 ^{a}	49.46 ± 0.03 ^{b}	<0.001
	6th day	44.85 ± 0.05 ^{f}	46.90 ± 0.03 ^{d}	45.93 ± 0.02 ^{f}	48.00 ± 0.03 ^{c}	53.87 ± 0.05 ^{a}	52.76 ± 0.03 ^{b}	<0.001
	12th day	44.93 ± 0.02	48.94 ± 0.04 ^{c}	46.10 ± 0.04 ^{e}	48.20 ± 0.04 ^{d}	54.17 ± 0.04 ^{a}	53.06 ± 0.03 ^{b}	<0.001
a_w	1st day	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	>0.05
	6th day	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.93 ± 0.01	0.92 ± 0.01	0.93 ± 0.01	>0.05
	12th day	0.93 ± 0.01	0.92 ± 0.01	0.93 ± 0.01	0.92 ± 0.01	0.92 ± 0.01	0.93 ± 0.01	>0.05

$^{a, b, c, d, e, f}$—Means within a row different superscript differ ($p < 0.05$).

The ripening time significantly affected ($p < 0.001$) the fat content, pH value, acidity and dry matter of sheep and goat cheese samples over the monitored period. Only the value of water activity unchanged ($p > 0.05$). Overall, physicochemical results were similar between sheep and goat cheese and reflected the composition of sheep and goat to the Valachian sheep and white shorthaired goat population that is normally used for cheese making.

3.2.2. Microbial Analyses

Time of ripening caused changes ($p < 0.001$) in the number of present bacteria in sheep and goat cheeses. Table 6 shows the mean value of detected bacteria during the ripening time of cheese analyses. The mean value of bacteria from *Enterobacteriaceae* family changed from 5.84 ± 1.14 log CFU/mL in examined sheep milk to 6.12 ± 0.82 log CFU/mL in sheep cheese on the first day of ripening. In goat cheese, the mean value of bacteria from *Enterobacteriaceae* family has increased from 5.68 ± 1.53 log CFU/mL in examined goat milk to 6.27 ± 087 log CFU/mL in goat cheese on the first day of ripening. No significant change ($p > 0.05$) in the number of bacteria from the family of *Enterobacteriaceae* occurred during the ripening time neither in sheep's cheese nor in goat's cheese. However, the number of these bacteria showed a declining trend during the ripening time.

Table 6. Means and their standard deviations (mean ± SD) of the selected bacteria (log CFU/mL) in samples of sheep and goat cheeses at 1st, 6th, and 12th days of ripening.

	Sheep Cheeses				Goat Cheeses			
	1st Day	6th Day	12th Day	*p* Value	1st Day	6th Day	12th Day	*p* Value
EB	6.12 ± 0.82	5.81 ± 1.09	5.49 ± 1.0	>0.05	6.27 ± 0.87	5.86 ± 1.07	5.32 ± 0.75	>0.05
CoPS	4.45 ± 0.64	4.77 ± 0.50	4.19 ± 0.13	>0.05	5.04 ± 0.43 ^{a}	4.41 ± 0.41	3.87 ± 0.78 ^{b}	<0.01
LAB	7.01 ± 0.70 ^{b}	8.32 ± 0.47 ^{a}	9.10 ± 0.45 ^{a}	<0.001	7.61 ± 0.24 ^{c}	8.58 ± 0.47 ^{b}	9.46 ± 0.48 ^{a}	<0.001

$^{a, b, c}$—Means within a row different superscript differ ($p < 0.05$; EB—*Enterobacteriaceae*; CoPS—coagulase-positive staphylococci; LAB—lactic acid bacteria.

In this study, the number of coagulase-positive staphylococci was detected, while in comparison with their number in milk samples, their amount was generally higher in sheep and goat cheeses. The number of coagulase-positive staphylococci increased in sheep's cheese compared to the number measured in milk as well as bacteria from *Enterobacteriaceae* family. Results showed that the mean value increased from 4.33 ± 0.57 log CFU/mL detected in sheep milk to 4.45 ± 0.64 log CFU/mL detected in sheep cheese during the first day of ripening. The same trend was observed in samples of goat's milk and cheese, where the mean value increased from 4.30 ± 0.56 log CFU/mL determined in goat milk

to 5.04 ± 0.43 log CFU/mL in goat cheese on the first day of ripening. No significant differences ($p > 0.05$) in the number of coagulase-positive staphylococci during the ripening time were found in sheep cheese samples. Contrarily, in goat cheese samples the numbers decreased from 5.04 ± 0.43 log CFU/mL set on the first day of ripening to 3.87 ± 0.78 log CFU/mL on the twelfth day of ripening ($p < 0.01$).

Lactic acid bacteria showed statistically significant differences ($p < 0.001$) in samples of sheep and goat cheeses. The mean value of lactic acid bacteria in samples of sheep cheese was 8.14 ± 1.03 log CFU/mL and in samples of goat cheese 8.55 ± 8.87 log CFU/mL (Table 6).

3.2.3. Color of Cheese

The ripening time of the cheeses significantly affected the overall color of the examined cheese samples. The mean values and standard deviation obtained for L*, a*, and b* parameters are shown in Table 7, which presents considerable changes during ripening time. In all tested samples of sheep's and goat's cheeses, no statistically significant difference ($p > 0.05$) was detected in the change of color properties due to the change in the month of analysis of the sample.

Table 7. Means and their standard deviations (mean ± SD) of color characteristics of sheep and goat cheeses at 1st, 6th, and 12th of ripening.

	Sheep Cheeses				Goat Cheeses			
	1st Day	6th Day	12th Day	*p* Value	1st Day	6th Day	12th Day	*p* Value
L*	$93.55^{a} \pm 1.76$	$92.68^{ab} \pm 1.93$	$91.61^{b} \pm 2.01$	<0.001	$88.91^{a} \pm 1.55$	$88.46^{a} \pm 1.57$	$87.16^{b} \pm 1.71$	<0.01
a*	$-3.16^{a} \pm 0.08$	$-3.02^{b} \pm 0.09$	$-2.88^{c} \pm 0.07$	<0.001	$-1.39^{a} \pm 0.05$	$-1.29^{a} \pm 0.10$	$-1.17^{b} \pm 0.08$	<0.01
b*	15.17 ± 1.24	15.29 ± 1.38	15.47 ±1.94	>0.05	17.06 ± 1.64	17.16 ± 1.21	17.35 ± 1.32	>0.05

$^{a, b, c}$—Means within a row different superscript differ ($p < 0.05$) L*—lightness; a*—green-red; b*—blue-yellow.

Differences in lightness, which changed from 93.55 ± 1.76 on the first day of ripening to 91.61 ± 2.01 on the 12th day of ripening ($p < 0.001$) were measured in sheep cheeses. The a* value has changed by ripening too. The most significant change was also detected between the 1st and 12th day ($p < 0.001$), while the differences between other days were less significant ($p < 0.05$). However, the b* value showed some increase, but the changes were not significant ($p > 0.05$).

The lightness value in goat cheeses showed a significant decrease from the 1st to the 12th day (88.91 ± 1.55 vs. 87.16 ± 1.71) ($p < 0.01$). The a* value increased between the same days as lightness decreased. The results showed change between the 3rd and the 12th day of ripening ($p < 0.01$) and between the 6th and the 12th day of ripening ($p < 0.05$). Sheep cheeses, as well goat cheeses, did not show significant changes in b* color.

4. Discussion

Our study was conducted to establish the quality of sheep and milk and cheese made from them produced on farm level. Physical and chemical analysis is an important tool for determining the quality of sheep and goat milk and dairy products. The influence of individual months on the qualitative characteristics of milk was recorded in studies by several authors [45,46]. The main goal of the study by Bhosale et al. [45] was to monitor the effect of individual months of lactation on the composition and physicochemical properties of milk. The individual months pointed out significant differences in fat content, which also agrees with the study by Charnobai et al. [47]. In their study, Merlin Junior et al. [48] recorded the mean values of fat (7.28%) and protein (5.86%) in sheep's milk, which correspond to our results in Table 1. Increase of protein and fat content in sheep's milk during lactation, was detected in the study of Vršková et al. [49], while the SCC, similar to our own study, remained practically unchanged throughout the lactation period. The difference detected in lactose levels between the various publications and our study can be explained by a worsening of udder health. It is well known that lactose levels are reduced during clinical

and subclinical mastitis which are also connected with high SCC [50,51]. The result of density value in this study is higher compared to results by Britio et al. [52] (1.036 mg/mL) and Simos et al. [53] (1.037 mg/mL).

Goat milk parameters, especially protein content (Table 2) were influenced by individual months. Average protein content of goat milk is 3.4% [3]. Our results showed higher value of detected protein in goat milk samples (Table 2). Increasing trend of fat content from the beginning till the end of lactation was confirmed by Vacca et al. [54] and Přidalová et al. [55] in goat milk. Same as in our case, other studies by Guo et al. [56], Strzałkowska et al. [57], and Mestawet et al. [58] found out the lowest fat content in goat milk in the mid lactation stage same as the results of our study (Table 2) during June and July. McInnis et al. [59] confirmed higher presence of fat and protein during the first month of milking. According to Goetsch et al. [2], who explained that the concentration of lactose in milk as well as the content of fat and proteins, can be affected by the months of milking. By Rolinec et al. [60] an average content of lactose present in goat's milk is 4.73%. The quality of goat milk fat and also protein is an important factor because it defines the ability of milk to be processed and has a relevant role in the nutritional and sensory quality of the products obtained from it [61]. The pH value (Table 2) was relatively stable during the whole lactation similar to the results by Kuchtík [62].

The evidence of high milk SCC emphasizes the need to find mastitis-control programs in order to improve milk hygiene. In Slovakia, only a few studies were done to examine the SCC in sheep milk in practical conditions. One large study was done by Tomáška et al. [63] who performed the bulk milk analysis collected in the summer season from March to August and revealed that only 7.3% of samples were in category below 500,000 cells mL^{-1}; while 49% of bulk milk samples were above 1,000,000 cells mL^{-1}. Jaeggi et al. [64], found out that the content of total protein was the lowest in milk with the highest SCC levels. However, other authors reported, that sheep milk with high SCC contains more total protein than milk with low SCC [65]. Furthermore, other authors, including us, agree that the SCC of goat milk is higher than that of cow milk, but goats may not suffer from mastitis. This observation also implies that there is not a very close relationship between the SCC of milk and the health condition of goats, contrary to cows [66].

The family of *Enterobacteriace* bacteria is considered as an indicator of hygienic conditions of milk production [67]. Our results agree with the results of other authors. The study of Muehlherr et al. [68] showed the maximum value of *Enterobacteriaceae* was 7.64 log CFU/mL in goat's milk and maximum value 5.34 log CFU/mL in ewe's milk. The count of coagulase-positive staphylococci in detected sheep milk and in goat milk (Table 6) did not reach 5.00 log CFU/mL, i.e., the volume of bacteria necessary for the production of an enterotoxin which is capable of inducing food-borne intoxication [69]. The number of *L. monocytogenes* detected in healthy sheep and goat milk is generally very low. Results correlate with other authors, Bogdanovičova et al. [70] in the study showed that *L. monocytogenes* was detected only in three samples of raw milk (1.3%) (0.6% cow's milk, 3.1% goat's milk, and 4.4% sheep's milk).

Dairy products are important sources of biological active compounds of particular relevance to human health such as lactic acid bacteria [71]. Lactobacilli are widely present in milk and various fermented dairy products. Their presence in raw cow, sheep, and goat milk was confirmed in the study by Výrostková et al. [72], where the number of bacteria of the genus *Lactobacillus* sp. was in raw cow's milk 3.8 $\pm$ 0.1 log CFU/mL, raw sheep's milk 3.2 $\pm$ 0.1 log CFU/mL, and in raw goat's milk 2.0 $\pm$ 0.1 log CFU/mL.

Physical and chemical properties of cheese quality (Tables 4 and 5) were affected by the months of milking and also by the ripening time of cheese. All measurements were not much different between sheep and goat cheese samples. Results of fat content, dry matter, and pH in sheep cheese samples in Table 4 is higher than in study by Murgia et al. [73] where the average detected fat content was 9.29 $\pm$ 3.02%, dry matter was 21.06 $\pm$ 5.61%, and pH value was 4.32 $\pm$ 0.07. Our results of physical and chemical indicators of goat cheese samples (Table 5) correspondent with study of goat cheeses made from organic

goat milk [74] where the value of fat content ranges from 25.3 to 32.9% and the value of pH ranges from 5.20 to 6.05. The results of dry matter in goat cheese (Table 5) were similar to results of goat's cheese in the Czech Republic where the average dry matter content was 46.83 ± 1.57%, with the range from 44.08% to 50.05% [16]. The acidity values support the safety of sheep and goat cheeses despite the relatively high-water activity values (0.93 ± 0.01), which is lower than the results by Janštová et al. [16].

For the consumer, the microbiological quality and safety of cheeses produced under traditional domestic conditions at the farm level depends primarily on the raw material used and its microbiological quality, on hygiene during production and on the possibility of subsequent contamination. Results of microbiological analyses of sheep and goat cheese are reported in Table 6. Microbial safety of the analyzed cheeses is affected by the bacteria [17,75].

Coliform bacteria such as bacteria from the family of *Enterobacteriaceae* are one of the major indicators of the condition of food production practice [76]. These bacteria slowly decreased during ripening (Table 6), which confirmed the results of Litopoulou-Tzanetaki and Tzanetakis [77], who present in their study that the level of *Enterobacteriaceae* depended on the time of cheese ripening; as the ripening time was extended, the levels of these bacteria decreased. The results of other authors demonstrate a high range in the level of coliform contamination in different types of cheese produced using traditional methods from cow's, goat's, sheep's, or mixed milk, from under 1 to 7.89 log CFU/mL [76].

The major representant of coagulase-positive staphylococci is *Staphylococcus* spp. which number can range from 3.54 to 6.50 log CFU/mL in traditional ripened cheeses produced from raw milk, depending on the ripening stage [77,78]. In the current study the presence of coagulase positive staphylococci ranges from 5.04 ± 0.43 log CFU/mL at first day to 3.87 ± 0.78 log CFU/mL on 12th day of ripening in goat cheese (Table 6). The levels of coagulase-positive staphylococci over 5 log CFU/mL can produce enterotoxins in an amount which can be dangerous for human health in general [79]. These microorganisms could be inhibited by highly competitive lactic acid bacteria which can survive changing conditions during the ripening time of cheeses [80,81].

B. cereus, *E. coli*, and *L. monocytogenes* were not found in our samples of sheep and goat cheese during the whole ripening time. Our results confirmed the results of other studies where *Listeria* spp. was not found in short-ripened cheeses produced in Poland and long-ripened cheeses produced in Brazil and Italy [82–84].

The main group of cheese microbiota consists of lactic acid bacteria, which are dominant from the beginning until the end of the process of ripening [18]. Overall, a high number of lactic acid bacteria (LAB) has been enumerated in the sheep and goat cheese samples on the 12th day of ripening (Table 6). Studies of other authors reported that lactic acid bacteria count in long-ripened cheeses was significantly higher (10.38 log CFU/mL) than that in short-ripened cheeses (8.30–8.45 log CFU/mL) [76]. Lactic acid bacteria in cheeses generally increase during the ripening process (Table 6) and play a significant role during the whole process of ripening [84]. They produce antimicrobial substances, and this is one of the reasons in this study why coagulase-positive staphylococci and bacteria from the *Enterobacteriaceae* family decrease during cheese ripening, when the number of lactic acid bacteria increased. Lactobacilli belong among aciduric bacteria, which are part of secondary flora. Lactic acid bacteria can produce bacteriocins, organic acids, and proteins, which are the antimicrobial substances, and they are capable of inhibiting the growth of pathogens [85]. Determination of antimicrobial potential of lactic acid bacteria isolated from Slovak raw sheep milk cheeses confirmed inhibitional potential of *Lactobacillus plantarum* strains against several foodborne pathogens, including *Staphylococcus aureus, Bacillus cereus*, *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes* [86].

5. Conclusions

Safety and quality of food—especially among milk and dairy products—is regulated by laws and regulations. A major part of this study was focused on the changes of physical,

chemical, and microbial properties of sheep and goat milk as well as the cheeses made from the analyzed milk during the ripening time.

The results of this study confirmed that the milking season of the Valachian sheep and white shorthair goats affected milk indicators and overall quality and safety of the dairy products such as cheeses. These results also support the production of sheep and goat cheese made from non-pasteurized milk. However, the evidence of high SCC levels in milk indicates a food safety risk associated with subclinical mastitis highlights the need to find mastitis control programs in flocks and herds to improve milk hygiene. Even though a number of studies have been carried out on the composition, quality, and safety of milk and dairy products, there is still not enough information of local studies concerning the nutritional composition of milk and the safety of dairy products produced. The results of this study can provide data which help in understanding the quality and safety of raw sheep and goat milk and cheeses produced on the farm level.

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