Research Article

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Factors influencing mastitis occurrence and milk quality in ewes: the role of seasonality and age

Abstract

The aim of this study was to evaluate the influence of seasonal factors and ewe age on mammary gland health and milk composition. Monitoring was conducted on a dairy flock of 200 Lacaune and East Friesian ewes located in central Slovakia. To evaluate the occurrence and etiology of mastitis, udder examinations and milk sampling for pathogen detection were performed three times during the grazing season: at the beginning, middle, and end. To compare milk production and composition in relation to age, 32 healthy ewes were selected during the same periods and divided into four groups based on their lactation number: 1st, 2nd, 3rd, and ≥ 4th lactation. The results showed that the prevalence of mastitis in the flock ranged from 7.7 to 11.0%, with subclinical forms at 4.5–8.5% and clinical forms at 2.0–5.0%. Regarding milk quality, ewes in the 2nd and 3rd lactations proved to be the most suitable, achieving the most stable and highest fat and protein content. Somatic cell count (SCC) increased with age and was more pronounced in ewes in their ≥ 4th lactation, where SCC reached 1,179 x 10^3. From a practical standpoint, it is most efficient for breeders to utilize ewes up to their third lactation, as they achieve peak productivity and an optimal SCC level. These findings can serve as a basis for optimizing herd management, planning flock replacement, and improving the overall quality of milk intended for further processing.

Keywords: ewes, breeding system, grazing, mastitis, lactation, milk quality

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Introduction

Sheep farming is one of the traditional sectors of agriculture in Slovakia and represents a significant component of the nation's cultural heritage. In the conditions of Central Slovakia, it is primarily implemented through extensive and semi-intensive farming systems. Breeding management uses both seasonal and out-of-season mating, employing methods such as individual hand mating or artificial insemination.[1]

In recent decades, sheep populations in Slovakia have declined; nevertheless, sheep milk production remains significant for food security and the overall economics of sheep farming.[2] Sheep milk is among the most nutritionally valuable types of milk. It is characterized by a yellowish hue, a typical aroma, and a sweet taste, and serves as a significant source of protein and minerals (Ca, P, and Zn). The composition of sheep milk differs markedly from both cow and goat milk. It contains approximately 20% dry matter, an average of 5% protein (80% of which is casein), and roughly 8% fat (Table 1). .Furthermore, milk contains various B-complex vitamins, enzymes (particularly amylases), amino acids, fatty acids, non-protein nitrogenous substances, hormones, cellular elements, and microorganisms.[3] The characteristic microbial, flavor, and aromatic properties of the milk are, to a certain extent, determined by its specific chemical composition, notably the distinct phospholipid content. Sheep milk is the primary raw material for producing sheep lump cheese, which is used to manufacture the most renowned product of Slovak sheep farming, bryndza.[4]

Table 1 Comparison of the main components of goat, sheep, and cow milk

Milk components (%)	Goat milk	Sheep milk	Cow milk
Fat	3,8	7,62	3,67
Non-fat solids	8,68	10,33	9,02
Lactose	4,08	3,7	4,78
Protein	2,9	6,21	3,23
Casein	2,47	5,16	2,63
Whey proteins	0,43	0,81	0,60
Ca/ Calcium	0,194	0,160	0,184
P/ Phosphorus	0,270	0,145	0,235
Cl/ Chloride	0,154	0,270	0,105
Vitamin A	39,0	25,0	21,0
Vitamin B1/Thiamine	68,0	7,0	45,0
Vitamin B12	210,0	36,0	159,0
Vitamin C	20,0	43,0	2,0
Vitamin D	0,70	ND	0,70

Source: Modified table according to Kováčová et al.[4]

Sheep milk production and quality are influenced by several factors, most notably nutrition, stage of lactation, ewe age, and udder health. Nutrient deficiencies can lead to a decline in milk yield, whereas grazing represents a natural source of high-quality nutrients.[5]

From a health perspective, inflammation of the mammary gland – mastitis is among the most serious diseases in sheep, as it reduces milk quality and causes economic losses for farmers due to the frequent culling of ewes. Mastitis is defined as a polyfactorial and polyetiological disease (Figure 1). Inflammation of the mammary gland can be caused by physical factors, such as various traumas, improperly adjusted milking equipment, or inadequate zoohygienic conditions.[6]

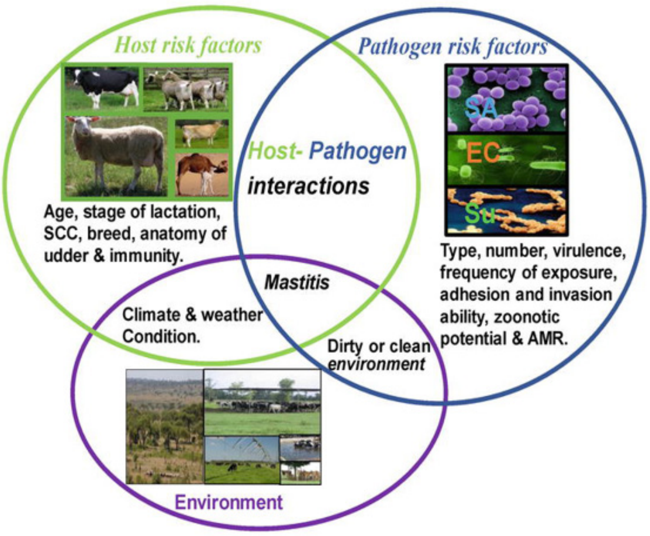

Figure 1 Risk factors for mastitis.

Note: SA - *Staphylococcus aureus*; EC - *Escherichia coli*; SU - *Streptococcus uberis*; SCC - somatic cell count; AMR - antimicrobial resistance.

Source: Dego[6]

Other causes may include toxic influences and thermal factors such as temperature and drafts. The most common cause of mastitis is infection. The causative agents enter the udder galactogenically (through the teat canal), traumatically (through trauma to the udder skin), and hematogenously. However, the most common route of entry of the etiological agent is the galactogenic route. Most often, only one half of the udder is affected. From an anatomical point of view, the duct system, represented by the alveoli, milk ducts, and milk cistern, may be affected. Furthermore, the interstitium and the walls of the milk ducts and cisterns may also be affected.[7]

In addition to direct milk loss, they increase costs for treatment, laboratory tests, and selection and culling of animals, and reduce animal lifespan. Subclinical forms, in particular, are often underestimated, but their cumulative effect has a significant economic impact on the farm. Reduced milk production negatively affects the growth and development of suckling lambs.[8] Prevention, early diagnosis, and proper management are therefore key to maintaining high milk quality and reducing the incidence of mastitis in ewes.[9]

Given the diversity of environmental and physiological factors affecting milk production, the objective of this study was to evaluate the incidence and etiology of mastitis in a flock of ewes during a grazing season, as well as to compare milk production and composition based on their age.

Material and methods

Monitored sheep farming

For the study, the 2024 grazing season was evaluated on a sheep farm located in central Slovakia near the city of Brezno. The farm has 200 sheep of the breed Lacaune and East Friesian and their crosses, which were monitored during one grazing season. The age composition of the monitored herd consisted of 65 sheep (32.5%) in the 1st lactation, 57 sheep (28.5%) in the 2nd lactation, 44 sheep (22.0%) in the 3rd lactation and 32 sheep (16.0%) in the ≥ 4th lactation. A semi-intensive farming system is mainly used for their breeding and grazing during the season. Ewes achieve a milk production of at least 200 to 300 L per lactation period, which requires not only a productive breed type but also appropriate nutrition.[10] Grazing is carried out near farms on intensive or semi-intensive pastures, which are regularly fertilized. The use of pastureland may involve shepherd supervision or the use of fenced enclosures. After grazing, the sheep are gathered back to the farm, where they are housed until the next day and machine-milked in an established milking parlor. In addition to modern milking technology, advanced feeding systems are used, including feeding wagons and feeding belts, with watering systems and supplemental feeding of sheep with silage enriched with grain concentrates. Particular attention in semi-intensive farming system is given to selection (performance and milk utility control), breeding (estrus synchronization is often used), and maintaining good animal health through effective prevention measures.

Examination of ewes and sampling

During one grazing season, the incidence of mastitis and the most important causative agents of this economically significant disease were monitored. All 200 ewes were comprehensively examined 3 times at three-month intervals (at the beginning, in the middle, and at the end of the grazing season), including clinical examination of the udder, assessment of CMT, and collection of milk samples. Before milk collection, all ewes underwent a clinical examination of the udder, including inspection and palpation, with a focus on shape, symmetry, consistency, soreness, temperature, and the presence of pathological changes (Figure 2). At the same time, sensory evaluation of milk and CMT from both halves of the udder was performed separately as a basic assessment of mammary gland health according to Vršková et al.[11]

Figure 2 Clinical examination of the mammary gland of ewes on a monitored farm.

Note: From left - Healthy udder and mammary gland with clinical mastitis.

In the event of a positive CMT score, milk samples were collected from the affected halves of the udder for bacteriological examination. MicroMast plates and blood agar were used to culture mastitis pathogens (Figure 3). Based on morphological features, the initial colonies grown on blood agar were re-cultured onto various selective media for an additional 24 hours at 37 °C. Identification of *Staphylococcus* spp. involved assessing growth on selective agars (5% blood agar, agar 110, Baird-Parker agar, Brilliance UTI Clarity Agar, OXOID Ltd, UK). Colonies were then identified by their shape, Gram stain, hemolysis type, catalase activity (3% H_2O_2, Merck, Darmstadt, Germany), esculin hydrolysis, and cytochrome C oxidase (Bactident Oxidase, Merck). Esculin-positive streptococci were cultured on modified Rambach agar for more precise identification of *Str. uberis* or

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Enterococcus spp., following the studies of Vasiľ et al.[12] and Holko et al.[13] Identification of each species using biochemical tests, specifically STAPHYtest 24, STREPTOtest 24, or ENTEROtest 24, and evaluated the results with the TNW ProAuto 7.0 software (Erba-Lachema, Brno, CZ), achieving over 90% accuracy in species identification.

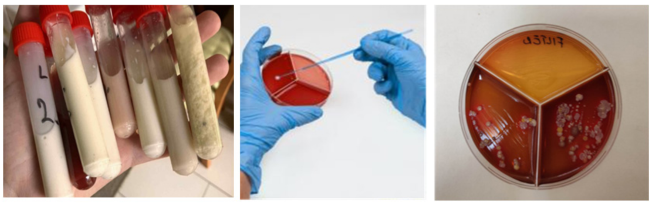

Figure 3 Milk samples and their cultivation on MicroMast plates.

Note: Milk samples were collected from the halves with a positive CMT score and cultivated on MicroMast plates for udder pathogens.

Culture samples were considered positive if one or more colony-forming units of main udder pathogens, such as *S. aureus, Str. dysgalactiae*, or *Str. agalactiae*, were detected. A sample was also considered positive if growth of a contagious udder pathogen was confirmed along with other environmental pathogens, or if these pathogens produced at least three CFU. If no major contagious pathogens were found or if three or more pathogens were isolated from a single milk sample, such cultures were deemed contaminated. Based on clinical examination of the mammary gland, assessment of CMT and laboratory culture of collected milk samples, the mammary gland was assessed as healthy, subclinical or clinical form of mastitis.[11]

Simultaneously, at the same intervals, 32 clinically healthy individuals were selected from the monitored ewes and divided into four groups (8 ewes per group) based on their lactation stage: 1st, 2nd, 3rd, and ≥ 4th lactation. Milk samples for the determination of milk composition were collected aseptically from the selected groups into sterile 50 ml tubes and transported at 4 °C to the laboratory for further analysis (Figure 3).

Determining the basic components of milk in samples from selected ewes as fat, protein and lactose content was tested by FTIR spectroscopy using MilkoScan FT 6000 (Foss Analytical A/S, Hillerød, Denmark), according to Záhumenská et al.[14] All examinations are accredited by SL Examinala by the Slovak National Accreditation Service according to ISO/IEC 17 025. Milk yield was determined from the amount of milk produced and the number of sheep milked during the individual monitoring periods.

Statistical analysis

In the statistical evaluation, the differences were compared in milk production and its qualitative parameters between the monitored groups of ewes. The results were evaluated using MS Excel (Microsoft, Redmond, Washington, USA). Basic statistical characteristics, such as mean, and standard deviation, were calculated for numerical data. Statistical analysis was performed by one-way analysis of variance. ANOVA and Tukey's test for multiple comparisons of means with a confidence interval set at 95% were performed using GraphPad Prism statistical software 8.3.0.538 (GraphPad Software, San Diego, CA, USA). The differences in mastitis occurrence, forms and the distribution of bacterial pathogens within the monitored periods were statistically analysed using the Chi-square test. The dependence of the individual signs was tested at a significance level α = 0.05.

Results

The results of the CMT assessment conducted on 200 ewes during the grazing season are presented in Table 2. During the initial examination (at the beginning of the grazing season), a positive CMT score was observed in 6.2% of the examined udder halves. Milk samples were collected from the affected halves, and a bacterial mastitis pathogen was identified in 19 of them. The lowest incidence of halves with a positive CMT score was recorded at the end of the milking season. Conversely, the highest number of CMT-positive halves and infectious pathogens was observed in the middle of the milking season (Table 2). During this period (mid-season), the highest incidence of clinical mastitis was recorded (5%). Subclinical forms occurred primarily at the beginning of the season (8.5%).

Table 2 Evaluation of CMT and infected halves of dairy sheep herds

Pasture Season	No. of examined sheep	Negative CMT		Positive CMT		Infected halves*	
		no.	%	no.	%	no.	%
Start	200	375	93.8	25	6.2	19	4.8
Middle	200	363	90.8	37	9.2	22	5.5
End	194	380	95	20	5	15	3.8

Note: CMT – California mastitis test performed on each half of the udder; Infected halves* - positive CMT with udder pathogen culture; a,b – values within the same column with different superscript letters differ significantly at P <0.05.

The lowest occurrence of clinical forms was recorded at the end of the season (2%; Figure 4), which was attributed to the culling of six ewes with clinical mastitis caused by *S. aureus* based on clinical manifestations and milk sample culture following the second sampling. Overall, the incidence of mastitis during the season ranged from 7.7% to 11.0%, with subclinical forms accounting for 4.5–8.5% and clinical forms for 2–5%.

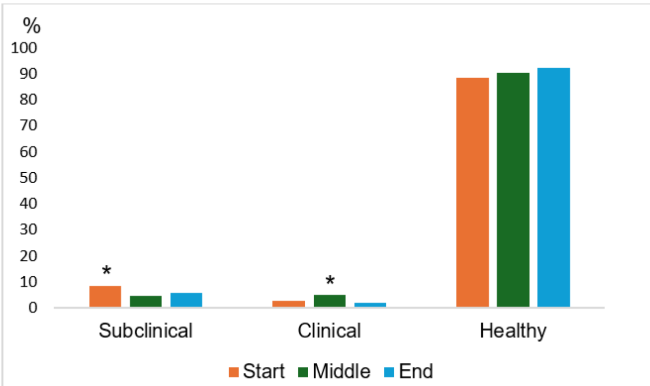

Figure 4 Occurrence of mastitis during pasture season.

Note: Chi-square test *p < 0.05.

The primary isolated pathogens were staphylococci, which accounted for up to 61.1% of all positive samples. *S. aureus* was isolated mainly from clinical mastitis cases, whereas coagulase-negative staphylococci (CNS) were predominantly isolated from subclinical forms. In addition to staphylococci, gram-negative bacteria such as *E. coli* and *Enterobacter* sp. were also isolated (Table 3).

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Table 3 Evaluation of bacterial mastitis pathogens from positive samples

Season	S. aureus no./%		CNS no./%		G^- bacteria no./%	
	SBK	Clinical	SBK	Clinical	SBK	Clinical
Start (19)	0/0	8/42.1	9/47.4	0/0	0/0	2/10.5
Middle (22)	2/9.1	5/22.7	12/54.5	0/0	1/4.5	2/9.1
End (15)	0/0	3/20.0	7/46.7	2/13.3	2/13.3	1/6.7
Total (56)	0	16/10.7	28/50.0	2/3.4	3/5.4	5/8.9

Note: SBK – subclinical mastitis; CNS – coagulase-negative staphylococci represented *S. warneri, S. chromogenes* and *S. epidermidis*; G^- bacteria – represented *E. coli* and *Enterobacter* sp.

The results of the second part of the study compare the influence of ewe age and lactation stage on the quantity and quality of sheep milk. It can be observed that at the onset of lactation in April, following the weaning of lambs, the daily milk yield (DMY) was highest across all monitored ewes, as the grazing quality was optimal during this period. Significant differences in the peak DMY at the beginning of the season were observed in the group of ewes in their second lactation (Figure 5).

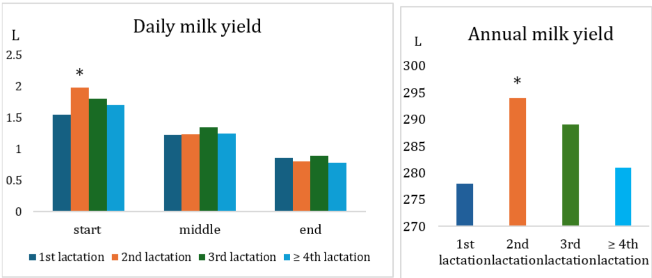

Figure 5 Comparison of milk production by lactation and season.

Note: Chi-square test *p < 0.05.

Subsequently, DMY experienced a moderate decline across all monitored groups, primarily attributed to climatic conditions affecting pasture quality (dry periods) as well as the physiological functions of the ewes (heat stress). Towards the end of lactation, DMY decreases due to the shorter lactation periods of certain individuals, particularly older ewes. The average annual milk yield (AMY) per lactation reached 285.5 kg of milk per ewe, with the highest AMY recorded in the group of ewes in their second lactation (294 L; Figure 5).

The milk composition analysis evaluated fat, protein, and lactose content, while somatic cell count (SCC) was assessed as a quantitative parameter (Table 4). The ewes were divided into four groups based on the number of lactations: 1st, 2nd, 3rd, and ≥ 4th lactation. The most stable values and lower SCC were observed in ewes during their 1st and 2nd lactations. SCC increased with advancing age in ewes, with the highest values recorded in those in their fourth or subsequent lactation. Regarding milk composition, the best results were found in ewes in their 3rd lactation, which achieved the highest fat content ($P > 0.05$; 6.43%). Other components were not significantly affected by ewe age (Table 4).

Table 4 Comparison of milk components by lactation

Parameter	1st lactation	2nd lactation	3rd lactation	≥ 4th lactation
Fat (%)	5.58±0.42a	5.85±0.38	6.43±0.54b	6.12±0.57
Protein (%)	4.25±0.28	4.23±0.35	4.52±0.32	4.01±0.25
Lactose (%)	5.15±0.29	5.22±0.26	5.72±0.30	5.41±0.32
SCC.103	232.6±31.1	183.5±23.7	531±42.3	1179±128.5

Note: SCC – somatic cell count; ^{a,b} – different letters in a row at statistical significance P>0.05.

Discussion

The highest incidence of mastitis was recorded during the first sampling at the beginning of the milking season, where subclinical forms predominated at a rate of 8.5%, caused by CNS. This result is consistent with the findings of Bergonier et al.,[8] which reported that the period following lamb weaning and the commencement of regular milking is the most critical time for the onset of intramammary infections.

According to Fthenakis,[15] coagulase-negative staphylococci are frequent causative agents of subclinical mastitis, which do not manifest clinically but increase the SCC, as they commonly colonize the skin of the udder. Similar results were observed in our study following the CMT evaluation. The CMT remains the first choice for sheep farmers in mastitis detection, reliably reflecting the SCC in milk samples. As the number of udder halves with positive CMT scores increased, so did the risk of intramammary infections. Given that after the initial examination, the farmer treated only ewes with the clinical form of mastitis, several individuals with subclinical forms progressed to the clinical stage. This became particularly evident during the second mid-season examination, when new clinical mastitis cases were recorded, peaking at 5%. At this point, 6 ewes exhibiting clinical mastitis caused by *S. aureus* were culled to reduce transmission and the contamination of other ewes.

According to Contreras et al.,[9] subclinical and persistent mastitis are frequent sources of staphylococci, which can be difficult to treat due to their potential for biofilm formation and antibiotic resistance. Environmental factors such as humidity, heat, and frequent microclimatic changes during grazing create favorable conditions for their growth and dissemination within the farm and milking parlor environment. Overall, during the entire milking season, clinical mastitis incidence ranged from 2% to 5%, with *S. aureus* being the causative agent in up to 80% of cases. This incidence is comparable to values reported for the Lacaune dairy breed, as described by Gonzalo et al.[16]

The objective of the second part of the study was to evaluate the influence of ewe age and lactation stage on the quantity and quality of sheep milk. At the beginning of the season, the DMY was highest across all monitored groups of ewes, as grazing quality peaked during April and May. An increased DMY was recorded in the group of ewes in their second lactation. Subsequently, DMY declined moderately across all monitored groups, primarily due to deterioration in the pasture stand during the warm months of June and July. In terms of milk quality, the best results were achieved by ewes in their 2nd and 3rd lactations, which is consistent with the study by Keresteš et al.[17] These ewes reached stable and also the highest values for fat content (in the 3rd lactation group; $P > 0.05$), protein, and lactose. From an economic perspective, they are the most suitable for milk production due to their superior performance and stable milk quality.

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According to Selvaggi et al.,[18] an inverse relationship between the volume of milk produced and the concentration of its constituents can be observed throughout the lactation curve. While a dilution effect occurs during peak lactation, resulting in a lower percentage of solid components, milk production declines towards the end of the season (from late summer to autumn), and its qualitative profile becomes richer. There is a notable increase in the content of fat, protein, and solids-not-fat, which is particularly desirable from an economic perspective due to higher technological yields in cheese production. Conversely, lactose levels remain relatively stable throughout the season, with only a slight decrease recorded in the final phase prior to the dry period.

Conclusion

The study confirmed a significant impact of seasonality on mastitis incidence, identifying the post-weaning period at the onset of the milking season as the most critical window for new intramammary infections. Staphylococci were established as the dominant pathogens, with *Staphylococcus aureus* consistently associated with severe, therapy-resistant clinical forms. To mitigate these risks, it is recommended that breeders implement routine antibiotic susceptibility testing after pathogen detection to ensure targeted and effective treatment.

Furthermore, a longitudinal analysis of milk quality revealed a positive correlation between ewe age and SCC. Ewes in their second and third lactations were identified as the optimal production cohort, yielding the highest concentrations of milk fat and protein while maintaining stable health parameters. These findings emphasize that maintaining high-quality milk production and reducing mastitis prevalence require a multifaceted approach that integrates strategic herd demography management, optimized grazing systems, and rigorous zoohygienic protocols throughout the entire production cycle.

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Conflicts of interests

The authors declare that there are no conflicts of interest.

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