INFLUENCE OF NUTRITIONAL SUPPLEMENTS ON MICROBIOLOGICAL COMPOSITION OF RAW SHEEP MILK

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
The aim of this study is to examine the effect of plant fat sources in sheep feed on somatic cells and the total number of microorganisms in raw milk samples obtained from 210 sheep, breed Pramenka in Una-Sana Canton. The research was conducted in three periods: winter, spring and summer. The herd was divided into experimental and control groups of sheep. In the experimental groups of sheep in each period of the study, omega-3 supplements were added to the diet, namely extruded flaxseed in the amount of 3.5%. The influence of added omega-3 supplements on the microbiological parameters of raw sheep milk, which was milked from experimental and control groups of sheep every fifteen days of the experiment in all periods of the study, was examined. Analysis of variance (ANOVA) found that during the winter and summer feeding period there is a statistically very significant influence of both experimental factors (treatment and period) on the total number of microorganisms and somatic cells in raw sheep milk (p <0.001). In the spring diet in somatic cells, factor treatment did not show a statistically significant effect (ns).

KEYWORDS: Sheep milk, microbiological analysis, somatic cells, total number of microorganisms

INTRODUCTION

Due to its specific composition, milk is a suitable environment for the growth and development of various bacteria. Milk from the farm can contain from several thousand to several million bacteria/ml, depending on the sanitary conditions on the farmstate that coliform bacteria are very often contaminants of raw milk, and the presence in milk indicates on fecal contamination as well as soil and water contamination[1]. When coliform bacteria are present in large numbers, they cause defects in the appearance and taste of milk, because they create gas and increase acidity.The basic indicators of the hygienic correctness of milk are the total number of microorganisms and the number of somatic cellsstate that the quality of milk is determined by its chemical composition, physical properties and hygienic correctness[2]. Milking is the most important process in milk production that affects the quality of milk. The milking has a significant influence on the daily amount of milk, the content of dry matter and milk fat, the freezing point and the hygienic quality of milk. Sanitary conditions during milking are especially important and affect the quality of milk[3].The number and type of microorganisms present in raw milk are determined by season, hygienic production conditions and diet.

MATERIALS AND METHODS

Two herds of sheep were selected for the research (a herd of pure Pramenka and a herd of crossed sheep) in the Una-Sana Canton. Groups of 35 experimental and 35 control sheep were formed in the herds, which were kept in identical conditions. In addition to the regular diet, the concentrates were added to the sheep in a daily amount of 300 g / head. A concentrate mixture with the addition of omega - 3 supplements (diet treatment) was added to the experimental group (Og) of sheep. In addition to the regular meal, a concentrate mixture without the addition of omega-3 preparations (diet treatment) was added to the control group of sheep (Kg). Experimental studieswere done during three different periods. The first period of winter (February and March, analyzes performed every 15 days - four sampling dates). The second period of the spring diet (May, analyzes done every 15 days - two sampling dates).

The third period of the summer diet (July, analyzes performed every 15 days - two sampling dates). Microbiological analysis of raw sheep's milk was

performed according to "Official Gazette of BiH" No. 21/11[4], where the number of somatic cells and the total number of bacteria was determined.The somatic cell number of raw sheep milk was determined on a „Fossomatic ™ Minor" instrument by the fluoro-opto-electronic method EN ISO 13366-2: 2006 / AC: 2007, IDT[5]. Electronic somatic cell counters work on the principle of epifluorescence microscopic technique. Somatic cells, after staining the nucleus (DNA) with fluorescent dye under ultraviolet light, fluoresce. This method is based on mixing a milk sample with a buffer and a staining solution.The total number of raw sheep's milk bacteria was determined on a BactoScan TM FC 50 instrument, by flow cytometry EN ISO 21187: 2004, IDT[6]. In the process of determining the bacteria are stained with fluorescent dye, then the individual components of the milk are sprayed so that the bacteria can be counted. The detector in the device registers fluorescent light emitted by the DNA of colored bacteria. Light impulses are converted into electronic impulses that are registered with a high-sensitivity pass-through detector.Analysis of variance (ANOVA) was used to examine the influence of factors (period and treatment) on the observed properties.

RESULTS AND DISCUSSION

Table 1. presents the results of statistical analysis of the value of microbiological properties of raw sheep's milk in the winter diet period (four times. Analysis of variance (ANOVA) found that there is a statistically very significant impact of both experimental factors (treatment and term) on the total number of microorganisms and somatic cells in raw sheep milk ($p < 0.001$) There was also a statistically very significant influence of factor interaction on somatic cells ($p < 0.001$), while for the total number of microorganisms, the level of significance of factor influence was 99% ($p < 0.01$).

Table 1. Results of statistical analysis of the average value of microbiological properties of raw sheep milk - period winter diet

Milk parameters		**Winter (february,march)**				**Level of significance (*p*)**		
		I(15)	**II (30)**	**III (45)**	**IV (60)**	period	treatment	Interaction
Somatic cells	Og	$557x10^3$	$183x\ 10^3$	$31\ x\ 10^3$	$105\ x\ 10^3$	***	***	***
	Kg	$85\ x\ 10^3$	$86\ x\ 10^3$	$58\ x\ 10^3$	$287x\ 10^3$			
Total number of microorganisms	Og	$108\ x\ 10^3$	$23\ x\ 10^3$	$27\ x\ 10^3$	$110x\ 10^3$	***	***	**
	Kg	$8\ x\ 10^3$	$26\ x\ 10^3$	$20\ x\ 10^3$	$70\ x\ 10^3$			

p– significance level: **p*<0,05; ***p*<0,01; *** *p*<0,001; ns –no statistical significance
Og – experimental group
Kg – control group
I, II, III, IV- periods of sampling(15, 30, 45, 60 day)

In our results in the experimental group of sheep, the number of somatic cells ranged from $31x10^3$ in the third sampling period to $557\ x\ 10^3$ in the first. The number of microorganisms was highest in the fourth term ($110\ x\ 10^3$) and lowest in the second ($23\ x\ 10^3$). Other values between (Og) and (Kg) as well as within the sampling period were in the values allowed by the legislation ("Official Gazette of BiH" No. 21/11)[4] but with significant variations both among herds and within the period.

In European countries, sheep milk can be processed into cheese if the number of bacteria is $<1\ x\ l0^6$ ml with pasteurization of the milk before processing. To make cheese from fresh milk, the number of bacteria must be <500,000 / ml, but the values of the number of somatic cells for sheep milk processing are not given[7].

The results of statistical analysis of the average value of microbiological parameters of raw sheep milk in the spring period of feeding (grazing) are shown in Table 2.

Table 2. Results of statistical analysis of the average value of microbiological parameters of raw sheep milk in the spring period of feeding

Milk parameters		Spring(grazing,may)		Significance level (*p*)		
		I(15)	II (30)	period	treatment	interaction
Somatic cells	Og	182 x 10^3	207x 10^3	***	ns	ns
	Kg	192x 10^3	103x 10^3			
Total number of microorganisms	Og	21x 10^3	15x 10^3	***	***	***
	Kg	11x 10^3	9x 10^3			

p– singificance level: **p*<0,05; ***p*<0,01; *** *p*<0,001; ns –no statistical significance
Os – experimental group
Ks – control group
I, II- periods of sampeling (15, 30 day)

Analysis of variance (ANOVA) it was determined that there was a statistically very significant influence of the factor (period) on the total number of microorganisms (p <0.001) in raw sheep milk, while in somatic cells the factor treatment did not show a statistically significant influence (ns). A statistically very highly significant influence of factor interaction on the total number of microorganisms was also found (p <0.001), while in somatic cells it did not show statistical significance (ns). Sheep milk, unlike cow's milk, contains significantly more microorganisms and mechanical impurities, which is conditioned by the way it is kept, fed and milked.

Microorganisms enter the milk from the udder or from the external environment (body, air, dishes, dust). There are a large number of microorganisms and impurities on the suction opening. Numerous microorganisms in milk can cause desirable changes, eg in cheese production (Lactobacillus spp., Lactococcus spp., Streptococcus spp.,). While harmful ones can cause various human diseases (Listeria, Salmonella, Brucella) or problems during the ripening of dairy products (Coliform , Psychrotrophs, Clostridium spp., Enterobacteriaceae)[8]. The obtained values between (Og) and (Kg) as well as within the sampling period were in the values allowed by the legislation ("Official Gazette of BiH" No. 21/11)[4] but with significant variations both among herds and within the period. The results of the statistical analysis of the average value of microbiological parameters of raw sheep milk in the summer grazing period are shown in Table 3.

Table 3. Results of statistical analysis of the average value of microbiological parameters of raw sheep milk in the summer grazing period

Milk parameters		Summer(grazing,july)		Significance level(*p*)		
		I-a	I-b	period	treatment	Interactio
Somatic cells	Og	680 x 10^3	362x 10^3	***	***	***
	Kg	814x 10^3	1274x 10^3			
Total number of microorganisms	Og	40x 10^3	159x 10^3	***	***	***
	Kg	45x 10^3	1707x 10^3			

p– significance level: **p*<0,05; ***p*<0,01; *** *p*<0,001; ns –no statistical significance
Os – experimental group
Ks – control group
I, II –periods of sampleing (15, 30 day)

Analysis of variance (ANOVA) revealed that there was a statistically very significant influence of both factors (period and treatment) on somatic cells and the total number of microorganisms (p <0.001) in raw sheep milk.A statistically very highly significant influence of factor interaction (p <0.001) on the total number of microorganisms and on the number of somatic cells was also determined. Direct comparisons of individual studies are not always possible because they depend on whether analyzes of individual or total milk samples have been performed[9]. The average number of somatic cells (SCC) determined in this study was 680 x 10^3, in the first period (summer), in the experimental herd, and in the control 814 x 10^3. The total number of microorganisms (CFU) in the experimental herd was 40 x 10^3 and in the control 45 x 10^3.The sampling date did not significantly affect the number of somatic cells in the milk, although higher

values were found in the second term[10], however, a significantly higher number of somatic cells was found in sheep milk of breed Pramenka, at the beginning than at the end of lactation. One of the causes of increased number of bacteria in milk can be bacterial inflammation of the udder, which causes a significant increase in the number of somatic cells above the physiological limit, and occurs due to the body's immune response to inflammation[11].

CONCLUSION

Based on the conducted tests on samples of raw sheep milk to whose food concentrate mixtures with omega-3 supplements in the form of extruded flaxeed and concentrate mixtures without the addition of the following conclusions can be drawn:

- Additions of omega-3 supplements to the diet of dairy sheep in the examined feeding periods (winter, spring and summer) did not have harmful effects on the microbiological composition of sheep milk.
- During the winter diet, it was found that there is a statistically very significant influence of both experimental factors (treatment and period) on the total number of microorganisms and somatic cells in raw sheep milk ($p < 0.001$).
- During microbiological analyzes of raw sheep milk in the diet in the spring period (grazing), it was found that there is a statistically very significant influence of factors (term) on the total number of microorganisms ($p < 0.001$), butsomatic cells the factor treatment did not show a statistically significant influence (ns).

In the summer diet (grazing) of sheep, it was found that there is a statistically very significant influence of both factors (period and treatment) on somatic cells and the total number of microorganisms ($p < 0.001$) in raw sheep milk.

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