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HYGIENIC QUALITY AND COMPOSITION OF RAW SHEEP'S BULK MILK SAMPLES ON SELECTED SLOVAK FARMS DURING YEAR 2018

Martina Vršková, Vladimír Tančin, Michal Uhrinčať, Lucia Mačuhová, Kristína Tvarožková

ABSTRACT

At the control of raw ewe's milk (REM) quality is a major microbiological criterion to the total bacterial count (TBC). The aim of our work was to determine the incidence of technologically important species of microorganisms in REM in Slovak Republic. At the monitored 28 ewe´s farms, we took bulk milk samples from evening or morning milking in spring, summer and autumn during year 2018. We analyzed nutrients (fat, protein, lactose and urea) and somatic cell count (SCC). We established technologically important microorganisms (MO) of psychotrophic MO, coliform MO, thermoresistant MO, spore-forming anaerobic MO. We have found a gradual increase in milk components, except for lactose, which is apparently related to the increasing cont of somatic cells during the milking period. We found that the TBC in raw sheep's milk complied an average of 132 x 10^3 CFU.mL^{-1} per spring (min 34 x 10^3 CFU.mL^{-1}, max 501 x 10^3 CFU.mL^{-1}), 300 x 10^3 CFU.mL^{-1} in summer (min. 31 x 10^3 CFU.mL-1, max 640 x 10^3 CFU.mL^{-1}) and in autumn with an average value of 147 x 10^3 CFU.mL^{-1} (min 52 x 10^3 CFU.mL^{-1}, max 276 x 10^3 CFU.mL$^{-1)}$. The enormous occurrence of psychrotrophic bacteria was found in one farm in northern Slovakia during spring and summer, in the summer we increased our number to 3 farms, in the autumn of 2 farms. At the other farms we evaluated the average value of 12 x 10^3 CFU.mL^{-1} per spring and 28 x 10^3 CFU.mL^{-1} in summer, 130.5 x 10^3 CFU.mL^{-1} in the autumn. The count of thermoresistent MO achieved 57 CFU.mL^{-1} per spring, 15 CFU.mL^{-1} in summer and 33 CFU.mL^{-1} in the autumn. The presence of spore-forming anaerobic MO in raw ewe´s milk was found during spring at six farms out of 15, but in the summer at just one in 9, in the autumn two farms.

Keywords: SCC; TBC; microbiological quality raw ewe´s milk; milk composition

INTRODUCTION

In Slovakia sheep farming is focused on milk production. The increase in dairy yield was ensured by imports of specialized milk breeds, lacaune or East Friesian sheep and their subsequent crossing with our sheep breeds (Tsigai, Improved Valachian) **(Tančin et al., 2013)**. Ewe’s milk was much more concentrated with about twice as much fat and 40% more protein that cow and goat milk. That also found that sheep milk responded differently in the cheese make procedure. It was more sensitive to rennet, coagulated faster, produced a firmer curd and yielded more cheese per unit of milk than cow milk **(Wendorff and Haenlein, 2017)**.

The quality of milk includes, in broad terms, the chemical composition, physical and technological properties, biochemical, microbiological and health indicators. In the narrower sense, we can only talk about hygienic (microbiological) aspects. Each of these features includes a number of quality features that determine the resulting quality of milk but also the quality of the dairy products. The quality of raw milk is regularly checked, because milk is the ideal environment for developing microorganisms because of its high water and nutrient content. All unwanted bacteria may not be pathogenic to humans. There are species that cause technological problems by producing thermostable lipolytic and proteolytic extracellular enzymes that pass through pasteurization in the active form. In order to avoid risks, and to ensure hygiene-sanitary quality and raw cows’, sheep’s and goats’ milk safety, in its **Regulations (EC) Nos. 852/2004** and **853/2004 European legislation** lays down general food hygiene rules and specific ones for food of animal origin. It also sets out aspects relating to mandatory controls (EC) No. 853/2004 on raw milk production on farms, and in dairy centres and laboratories. Raw milk has to be tested for not only its physico-chemical composition, but also for its hygienic characteristics, such as microbiology, somatic cell count (SCC) **(Martínez et al., 2018)**. According to which the total bacterial count (TBC) in 1 mL of raw sheep's milk (at 30 °C) must not exceed 1 500 000 CFU and for raw milk for further processing not subjected to heat treatment, this number is reduced to 500 000 CFU. The TBC in the raw sheep's milk delivered indicates the overall level of breeding hygiene and technology of harvesting (machine and hand milking) and milk storage. TBC reflects the hygiene of breeding conditions in milk production and is in the hands of the breeder itself. Bacterial contamination comes from a variety of sources, such as flora and pathogens present in hives, milking facilities, during storage and transport, feeding, rinsing water, udder or mastitis milk. Some of these bacteria are resistant to pasteurization or are able to grow at refrigeration temperature or to indicate fecal contamination, mastitis, or they can ferment lactic acid to butter, CO_2 and H_2, which cause late flushing of the cheese **(Gonzalo, 2017)**.

Scientific hypothesis

The occurrence of individual species of technologically significant microorganisms is influenced by the hygiene of obtaining milk in the milking process.

MATERIAL AND METHODOLOGY

At the monitored 28 ewe´s farms, we took bulk milk samples from evening or morning milking in March, April and May (spring), in June, July and August (summer) and September, October, November (autumn) during year 2018. Nutrients (fat, protein and lactose) were analyzed using the MilkoScan FT 120 (Foss Electric, Hillerød, Denmark). Somatic cell count (SCC) were set on the Somacount 150 (Bentley Instruments, Chaska, MN, USA). Urea was determined by polarimetry method. We analyzed the total bacterial count (TBC, mandatory indicator according to EC Regulation No. 1662/2006) according to **STN ISO 4833:1997**. We established technologically important microorganisms (MO) of psychotrophic MO according to **STN ISO 6730:2000** and coliform MO according to **STN ISO 4832:1997**. The presence of thermosensitive MO was detected in the Plate-Count-Agar and the presence of spore-forming anaerobic MO by liquid paraffin irrigation.

Statistic analysis

The values were evaluated through mean and standard deviation by Microsoft Excel 2013.

RESULTS AND DISCUSSION

It is known that the fat and protein content of milk is dependent on nutrition, and indirectly, nutrition will also affect the solids-non-fat (SNF) of milk. In Table 1 are presented the basic composition of milk and non-fat dry matter during the milking period. We have found a gradual increase in milk components, except for lactose, which is apparently related to the increasing number of somatic cells during the milking period and consequently the health of the milk udders.

Both fat and protein tend to increase throughout the lactation as well as **Kuchtík et al. (2017)**. This would typically result in higher cheese yields in late lactation milk **(Wendorff and Haenlein, 2017)**. As the SCC increases in the milk supply, the composition of milk also changes. As SCC increased, milkfat and the Casein/Total Protein ratio decreased. Protein recovery rate was lower in the high SCC milk while cheese yield was not significantly different.

Bocquier and Caja (2004) are reported that a high level of nutrition will reduce the level of milkfat but increase milk protein and casein. Conversely, a negative energy balance will decrease milk protein and increase milkfat. Milk protein will increase with an increased level of dietary protein. When feeding higher levels of concentrate in the diet, milkfat will be decreased and milk protein will be increased. The degree of impact from nutrition of the ewe will obviously be limited by the potential milk production capacity of the animal dictated by genetics. These trends are consistent with our results. Urea content depended on feed intensity, feeding system and pasture quality.

In Table 2, we presented the species of the most important technological types of bacteria. We found that the TBC in raw sheep's milk complied with the requirements of **Commission Regulation No. 1662/2006** with an average of 132 x 10^3 CFU.mL^{-1} per spring (min 34 x 10^3 CFU.mL^{-1} and max 501 x 10^3 CFU.mL^{-1}),

Table 1 Milk composition during the milking period.

Season	SNF		Milk composition (%)						Urea	
			fat		protein		lactose		%	
	mean	St.deviation	mean	St.deviation	mean	St.deviation	mean	St.deviation	mean	St.deviation
spring	11.26	0.59	7.50	1.61	5.56	0.58	4.84	0.45	42.39	12.44
summer	11.65	0.32	7.91	0.85	5.95	0.32	4.81	0.15	61.82	7.75
autumn	11.74	0.53	8.69	0.29	6.52	0.30	4.29	0.54	55.87	7.62

Note: SNF – solids non-fat.

Table 2 Hygienic quality of raw sheep's milk.

Microbiological characteristics (x 10^3 CFU.mL^{-1})	spring (n = 15)		summer (n = 9)		autumn (n = 4)	
	mean	St.deviation	mean	St.deviation	mean	St.deviation
TBC	132.13	87.71	300.00	217.01	147.00	124.78
Psychrotrophs MO	12.33	40.87	33.86	3.87	13.05	4.50
Coliforms MO	0.40	-	3.61	-	0.30	-
Termorezistant MO v 1 mL	57.69	29.31	13.33	11.15	40.50	33.27
SCC (x 10^3.mL^{-1})	1229.93	818.40	1411.08	770.25	2468.25	1147.14

Note: TBC – total bacteria count, SCC – somatic cell count.

300 x 10^3 CFU.mL^{-1} in summer (min. 31 x 10^3 CFU.mL^{-1} and max 640 x 10^3 CFU.mL^{-1}) and in autumn with an average value of 147 x 10^3 CFU.mL^{-1} (min 52 x 10^3 CFU.mL^{-1} and max 276 x 10^3 CFU.mL$^{-1)}$. **Gonzalo (2017)** found similar TBC to our spring, **Martínez et al. (2018)** significantly lower values (49 x 10^3 CFU.mL^{-1}). **Vršková et al. (2017)** reported in the summer 2016 TBC range of 187 to 964 x 10^3 CFU.mL^{-1}. **Skapetas et al. (2017)** found a higher TBC of 494 x 10^3 CFU.mL^{-1} by SCC 313 x 10^3 cells in 1 mL. **Kondyli et al. (2012)** found lower TBC values in summer of 170 x 10^3 CFU.mL^{-1} than in the spring of 600 x 10^3 CFU.mL^{-1}. The microbiological quality of sheep's milk according to **Gamčíková and Hanzelyová (2009)** in the primary production is mainly affected by unmasked mastitis of ewes. **Carloni et al. (2016)** found a range between the farms at TBC of 2 to 865 x 10^3 CFU.mL^{-1} and SCC from 151 to 3384 x 10^3 cells in 1 mL. **Kološta and Drončovský (2006)** found an arithmetic mean TBC of 21.921 x 10^3 CFU.mL^{-1} of raw sheep's milk. **Ducková and Čanigová (2004)** determined the TBC from 57 x 103 to 3,400 x 103 CFU.mL^{-1} at an average of 580 x 103 CFU.mL^{-1}.

The somatic cells count (SCC) is not yet a mandatory indicator as it is for dairy cows. In the spring, 7 farms of 15 had SCC above 1000 x 10^3 cells in 1 mL. The remaining farms ranged from 131 to 825 x 10^3 cells in 1 mL. In the summer, SCC was in 5 farms over 1000 x 10^3 cells in 1 mL. The remaining four farms ranged from 60 to 965 x 10^3 cells in 1 mL. In the autumn there were 2 farms out of 4. The remaining two farms reached SCC 958 x 10^3 cells per 1 mL. **Martínez et al. (2018), Kuchtík et al. (2017)** and **Gonzalo (2017)** reported lower SCC than our results.

Season was an important effect associated with the variation of bulk tank milk prevalence for specific bacterial groups and pathogens. Psychrotrophic and coliform bacterial groups were highest in connection with more dirty beds and udders due to the wetter weather (ambient combinantion) and with beginning of milking season **(Gonzalo, 2017)**. Raw milk is stored in the primary production at 8 °C and can result in the growth of psychrotrophic microflora. Its proven relationship with a high incidence of lipolytic and proteolytic activities on milk and cheese components.

The enormous occurrence of psychrotrophic bacteria was found in one farm in northern Slovakia during spring and summer, in the summer we increased our number to 3 farms, in the autumn of 2 farms. We did not, therefore, enter statistical evaluation. We explain this by contaminating the milk in insufficiently disinfected and cooled collecting containers in accordance with statement **Ducková and Čanigová (2004)**. The remaining farms have a milking parlor and beside the dairy tank with cooling. At the other farms we are evaluated the average value of 12 x 10^3 CFU.mL^{-1} per spring and 28 x 10^3 CFU.mL^{-1} in summer, 130.5 x 10^3 CFU.mL^{-1} in the autumn. **Ducková and Čanigová (2004)** found up to 240 x 10^3 CFU.mL^{-1} psychrotrophic MO.

Thermodurics are a contaminant group of milk that contains thermophilic spore-forming bacteria which can survive pasteurization during dairy-product processing causing dairy-product spoilage in the post-processing **(Gonzalo, 2017)**. The count of thermoresistent MO achieved 57 CFU.mL^{-1} per spring, 15 CFU.mL^{-1} in summer and 33 CFU.mL^{-1} in the autumn. **Gonzalo (2017)** found a high incidence of thermoresistent MO (930 CFU in 1 mL) by the ewes.

Several studies in ewe bulk tank milk showed that the main on-farm management risk factors associated to an increase of spore counts were farm-made total mixed ration, the silages and wet brewer's grains used for feeding, and the presence of dust in the milking parlour **(Arias et al., 2013)**. The presence of spore-forming anaerobic MO in raw ewe´s milk was found during spring at six farms out of 15, but in the summer at just one in 9, in the autumn the count rose to two farms.

CONCLUSION

The amount of microorganisms in milk gives us an overall picture of the level of hygiene in the primary production. The degree of contamination of raw cows' milk with mesophilic and psychrotrophic microorganisms affects the dairy health and hygiene of dairy ewes, the hygiene of the milkers and the environment in which the ewes are farmed and milked, the methods used for the preparation of the udder and the milking technique, the methods used for cleaning and sanitizing milking equipment and bulk tank milk. Depending on the species of microorganisms found in milk, we can identify the source of contamination and then use the correct methods to eliminate them. For small ruminants, milk hygiene is important for serious economic and sanitary consequences for farmers, the processing industry and consumers due to the interrelationship between loss of production, yield in cheese production, excreted milk (and its safe disposal) and consequently the safety of dairy foods for the consumer. Consumers' demands on "natural" food, heat untreated, added preservatives or increased salt concentration are increasing. Such foods also include raw milk.

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Contact address:

*Ing. Martina Vršková, PhD., National Agricultural and Food Centre, RIAP Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic, Tel.: +42137 6546264,
E-mail: vrskova@vuzv.sk

Prof. Vladimír Tančin, DrSc., National Agricultural and Food Centre, RIAP Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Veterinary Sciences, Trieda A. Hlinku 2, 949 76 Nitra, Slovak Republic, Tel.: +42137 6546153,
E-mail: tancin@vuzv.sk
ORCID: https://orcid.org/0000-0003-2908-9937

PaeDr. Michal Uhrinčať, PhD., National Agricultural and Food Centre, RIAP Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic, Tel.: +42137 6546162,
E-mail: uhrincat@vuzv.sk
ORCID: https://orcid.org/0000-0002-5378-617X

Ing. Lucia Mačuhová, PhD., National Agricultural and Food Centre, RIAP Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic, Tel.: +42137 6546171,
E-mail: macuhova@vuzv.sk
ORCID: https://orcid.org/0000-0002-9624-1348

Ing. Kristína Tvarožková, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Veterinary Sciences, Trieda A. Hlinku 2, 949 76 Nitra, Slovak Republic, Tel.: +421944 385272, E-mail: kristina.tvarozkova@uniag.sk

Corresponding author: *