

CHANGES IN THE MICROFLORA COMPOSITION OF GOAT AND SHEEP MILK DURING LACTATION

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ABSTRACT

The aim of this work was to determine extend of microbial contamination of raw milk in individual seasons. Raw goat milk (3 farms) and sheep milk (2 farms) were analyzed. Milk was produced on farms of different way of farming and with a different number of milked animals. Samples were taken during lactation three terms in the beginning, middle and end of lactation. In milk, following groups of microorganisms were determined by standard methods: total count of microorganisms (TCM), psychrotrophic microorganisms, *Enterobacteriaceae*, lactic acid bacteria (lactobacilli), enterococci, aerobic and anaerobic thermoresistant microorganisms (TMRae, TMRan), micromycetes (yeast and moulds). In goat milk, the following numbers of microorganisms were detected: total count of microorganisms (TCM) from 10^5 to 10^9 CFU x mL⁻¹, lactobacilli from 10^2 to 10^5 CFU x mL⁻¹, bacteria fam. *Enterobacteriaceae* from 10^1 to 10^5 CFU x mL⁻¹, enterococci from 10^1 to 10^5 CFU x mL⁻¹, thermoresistant aerobic and anaerobic microorganisms (TMRae and TMRan) from units to 10^3 resp. 10^5 CFU x mL⁻¹, psychrotrophic microorganisms from 10^1 to 10^6 CFU x mL⁻¹, micromycets from 10^1 to 10^4 CFU x mL⁻¹. In the sheep milk, the following numbers of microorganisms were determined: TCM from 10^5 to 10^6 CFU x mL⁻¹, lactobacilli from 10^3 to 10^6 CFU x mL⁻¹, bacteria fam. *Enterobacteriaceae* from 10^1 to 10^5 CFU x mL⁻¹, enterococci from 10^1 to 10^4 CFU x mL⁻¹, TMRae and TMRan from units to 10^5 CFU x mL⁻¹, psychrotrophic microorganisms from 10^4 to 10^6 CFU x mL⁻¹, micromycets from 10^2 to 10^4 CFU x mL⁻¹. From the above mentioned results, the following conclusions can be suggested. The bacterial counts of raw goat and sheep milk are highly variable and influenced by a number of important factors in the course of lactation and year (temperature, health, secondary contamination etc.). The bacterial numbers are not affected by the stage of lactation. High numbers of microorganisms in goat and sheep milk may be primarily caused by the insufficient cleaning and sanitizing of milking equipment or low hygiene of hand milking. An important role may also act the cooling rate of milk and purity of cooling equipment.

Keywords: goat milk; sheep milk; microflora of raw milk; total count of microorganisms

INTRODUCTION

Milk due to its beneficial composition and properties is suitable environment for the development of contaminating microorganisms. A relatively low number of microorganisms is contained in the milk of healthy animals mainly tending to settle in the teat canal and passes into the milk tank of udder. The number of microorganisms in milk ranges from 10^1 to 10^3 respectively 10^4 CFU x mL⁻¹ in the process of the leaving udder. A 10^5 CFU x mL⁻¹ could be obtained in the milk from the animal with the textured flaccid sphincter. The bacteria of the genus *Micrococcus*, *Enterococcus*, coryneform bacteria are represented, and infrequently e.g. staphylococci. In the diseased animals, e.g. *Streptococcus agalactiae*, *S. dysgalactiae*, *Staphylococcus aureus*, *E. coli*, and *Klebsiella* etc. can also occur (Hejlová, 1997; Görner and Valík, 2004). During milking, the milk is exposed to secondary contamination from the environment of the milking equipment, hands of the milker, and udder etc. (Hejlová, 1997; Malá et al., 2010). The quality of milk hygiene is microbiologically significant, which varies in terms of the types and numbers according to climate,

weather, grazing, lactation, housing, health, herd management etc. According to Švejarová et al. (2010), inferior microbiological parameters may prove a negative effect on the final product. When the bacteria content is too low, not only pathogenic microflora can be disturbed but also the natural non-pathogenic one, which can significantly affect the properties of dairy products made from raw milk (Kalantzopoulos, 2003).

The criteria for hygienic quality of raw milk are listed in the Regulation of the European Parliament and Council Regulation (EC) no. 853/2004 as amended. The microorganism content at 30 °C (per mL) should be ≤ 1500000 in goat and sheep milk (rolling geometric mean over a two-month period, at least two samples per month). However, if the milk is intended for the production of dairy products from raw milk by a process without heat treatment, the milk should contain $\leq 500\ 000$ microorganisms per mL. These regulations are the only applicable microbiological criteria in the Czech Republic for raw goat and sheep milk.

The aim of this study was to find out the dynamics of the microflora composition of raw goat and sheep milk from different farm breeds during lactation.

MATERIAL AND METHODOLOGY

Raw goat milk (3 farms) and sheep milk (2 farms) were analyzed. Milk was produced on farms of different way of farming and with a different number of milked animals (Tab. 1 and 2). Samples were taken during lactation in the beginning (spring), middle (summer) and end of lactation (autumn). In milk, following groups of microorganisms were determined by standard methods: total count of microorganisms (TCM) on PCA-AB medium (MILCOM – Tábor, Czech Rep.) at 30 °C for 72 h, psychrotrophic microorganisms on PCA medium (MILCOM – Tábor, Czech Rep.) at 6.5 °C for 10 days, fam. *Enterobacteriaceae* on VRBG medium (MILCOM – Tábor, Czech Rep.) at 37 °C for 24 h, lactobacilli on MRS medium (MILCOM – Tábor, Czech Rep.) anaerobic cultivation at 37 °C for 48 h, enterococci on Slanetz-Bartley Agar (Merck, Germany) at 37 °C 48 h, aerobic and anaerobic thermoresistant microorganisms (TMRae, TMRan) after thermoinactivation (85 °C 10 min.) on PCA at 37 °C for 48 h, micromycetes (yeast and moulds) on YGC medium (MILCOM - Tábor, Czech Rep.) at 25 °C for 120 h. After the cultivation of particular Petri dishes accrued colonies were counted and the result was expressed in CFU x mL⁻¹.

RESULTS AND DISCUSSION

The results of microbiological analyzes of goat and sheep milk samples are shown in Table 3. In goat milk, the

following numbers of microorganisms were detected: total count of microorganisms (TCM) from 10⁵ to 10⁹ CFU x mL⁻¹, lactobacilli from 10² to 10⁵ CFU x mL⁻¹, bacteria fam. *Enterobacteriaceae* from 10¹ to 10⁵ CFU x mL⁻¹, enterococci from 10¹ to 10⁵ CFU/mL, thermoresistant aerobic and anaerobic microorganisms (TMRae and TMRan) from units to 10³ resp. 10⁵ CFU x mL⁻¹, psychrotrophic microorganisms from 10¹ to 10⁶ CFU x mL⁻¹, micromycets from 10¹ to 10⁴ CFU x mL⁻¹. In the sheep's milk, the following numbers of microorganisms were determined: TCM from 10⁵ to 10⁶ CFU x mL⁻¹, lactobacilli from 10³ to 10⁶ CFU x mL⁻¹, bacteria fam. *Enterobacteriaceae* from 10¹ to 10⁵ CFU x mL⁻¹, enterococci from 10¹ to 10⁴ CFU x mL⁻¹, and TMRae and TMRan from units to 10⁵ CFU x mL⁻¹, psychrotrophic microorganisms from 10⁴ to 10⁶ CFU x mL⁻¹ micromycets from 10² to 10⁴ CFU x mL⁻¹.

TCM is only one legislative covered group of microorganisms in milk of small ruminants. The numbers are regulated by the European Parliament and Council Regulation (EC) no. 853/2004 and the maximum of ≤1.5 million cells should be contained in 1 mL. The average values of two-year monitoring for individual farms and lactation period are shown in Fig. 1. The results show that the limit value was multiply exceeded in the monitoring period for most of the farms. The exception is the farm no. 1, for which the limit value is not exceeded in any single case and TCM values ranged from 10⁴ to 10⁵ CFU x mL⁻¹. This state of farm no. 1 is held in the long term (Kalhotka et al., 2013). The observed values of TCM of farm no. 1 prove to be very close to the average values of 1.1 x 10⁵ CFU x mL⁻¹ as indicated Kouřimská

Table 1 Chacterization of farms.

Farm	Typ of milk	Breed	Type of farming	Milking animals	Region
1	goat	White Shorthaired Goat	conventional	131	dist. Blansko (SMR)
2	goat	White Shorthaired Goat	conventional	18	dist. Prostějov (OR)
3	goat	Crossbreed of Anglo-Nubian goat	organic	20	dist. Vyškov (SMR)
4	sheep	Lacaune	conventional	83	dist. Šumperk (OR)
5	sheep	Lacaune	organic	100	dist. Vsetín (ZR)

dist. – district, SMR – South Moravian Region, OR – Olomouc Region, ZR – Zlín Region

Table 2 Selected parameters relating to milking on farms.

Farm	Milking per day	Toilet m. gland	Parlor	Milking post-treatment	Cooling equipment
1	2x	wet	parallel	no	cooling equipment
2	2x	wet	parallel	barrier	spec. glasses in the refrigerator
3	2x	wet	spec. box	no	spec. glasses in the refrigerator
4	2x	wet	parallel	barrier	cooling equipment
5	2x	dry	circular	no	cooling equipment

and Dvořáková (2008). TCM fluctuated during lactation between the farms and it can not be clearly said that TCM rose with the increasing duration of lactation. At the same time, significant annual differences were also found out up to two logarithmic orders. Nevertheless, the individual

milk samples obtained from healthy animals contained small numbers of microorganisms, as for instance mentioned Králíčková et al. (2011), TCM can achieve high numbers in mixed samples. The average values of TCM for the whole period are shown in Fig. 2. Even here,

Table 3 Counts of microorganisms in goat and sheep milk in CFU x mL⁻¹.

Farm	Seasons	Year	TCM	Lbc.	Entero.	Ent.	TMRae	TMRan	Psych.	Mikromyc.
Farm 1	spring	2013	2.8×10^5	3.7×10^3	2.0×10^3	3.5×10^2	11	1	2.0×10^4	2.1×10^3
		2014	2.1×10^5	4.6×10^3	7.1×10^3	22	28	23	1.5×10^5	1.9×10^3
	summer	2013	2.3×10^5	1.2×10^4	1.6×10^3	4.4×10^2	23	28	8.4×10^4	2.9×10^3
		2014	9.5×10^5	3.2×10^5	1.6×10^4	1.7×10^2	24	21	2.6×10^5	2.5×10^3
	autumn	2013	7.0×10^4	8.6×10^3	30	2.5×10^2	9.8×10^3	4.2×10^3	3.7×10^3	9.1×10^2
		2014	7.7×10^5	3.9×10^2	2.7×10^4	6.6×10^2	2	1	2.2×10^5	1.6×10^3
Farm 2	spring	2013	2.3×10^7	9.5×10^5	5.6×10^3	1.2×10^5	2.9×10^3	9.8×10^2	4.7×10^6	1.9×10^4
		2014	9.5×10^5	2.4×10^3	3.2×10^2	8.7×10^2	1.4×10^5	20	2.3×10^5	1.2×10^3
	summer	2013	1.1×10^5	2.7×10^2	11	2.0×10^2	8	1	3.5×10^3	1.6×10^2
		2014	1.7×10^6	1.0×10^5	3.8×10^4	1.7×10^4	23	16	4.3×10^5	6.9×10^3
	autumn	2013	1.1×10^7	8.4×10^5	7.2×10^4	1.6×10^5	7.8×10^3	82	1.1×10^6	3.2×10^3
		2014	3.0×10^6	1.4×10^3	6.9×10^3	7.7×10^2	5.6×10^2	3.8×10^2	2.1×10^5	1.0×10^4
Farm 3	spring	2013	7.1×10^5	6.0×10^3	3.6×10^3	61	5	0	1.2×10^6	1.6×10^3
		2014	1.4×10^5	5.3×10^3	3.9×10^2	94	20	2.2×10^2	5.1×10^3	65
	summer	2013	2.3×10^5	3.0×10^2	2.6×10^2	32	3	1	<100	23
		2014	5.2×10^6	5.4×10^4	9.2×10^5	3.9×10^3	1.1×10^2	90	1.4×10^4	1.5×10^3
	autumn	2013	1.2×10^9	2.1×10^3	7.9×10^2	5.4×10^2	10	15	7.0×10^3	2.7×10^3
		2014	3.7×10^7	1.3×10^4	1.6×10^4	6.0×10^4	3.3×10^2	92	1.5×10^6	7.8×10^2
Farm 4	spring	2013	3.8×10^6	9.2×10^4	4.5×10^4	3.8×10^2	58	1.2×10^2	1.3×10^6	1.7×10^4
		2014	5.3×10^6	1.1×10^5	6.6×10^3	1.6×10^3	5.1×10^3	3.6×10^3	1.6×10^6	5.7×10^3
	summer	2013	3.8×10^5	1.4×10^5	4.7×10^3	2.2×10^3	15	9	1.6×10^4	1.1×10^4
		2014	8.2×10^5	1.3×10^5	4.6×10^4	1.8×10^4	32	20	6.7×10^5	3.5×10^4
	autumn	2013	5.9×10^6	2.2×10^3	2.1×10^5	5.4×10^2	1.1×10^5	1.5×10^5	1.2×10^6	5.0×10^4
		2014	2.5×10^6	6.5×10^3	6.8×10^3	1.4×10^4	90	7.1×10^2	6.6×10^5	4.8×10^4
Farm 5	spring	2013	4.5×10^5	1.8×10^3	4.0×10^3	95	48	5	1.3×10^4	1.6×10^2
		2014	3.7×10^6	1.9×10^4	<100	9.5×10^3	8.0×10^2	13	3.5×10^4	3.4×10^2
	summer	2013	1.1×10^6	1.8×10^4	1.4×10^3	2.2×10^4	8	11	4.5×10^4	4.9×10^2
		2014	4.0×10^6	2.3×10^6	3.8×10^4	9.9×10^4	2.1×10^3	57	8.1×10^5	1.7×10^3
	autumn	2013	5.2×10^6	1.1×10^6	2.3×10^3	1.1×10^4	5.3×10^2	4.0×10^2	2.2×10^6	2.5×10^4
		2014	4.5×10^6	1.8×10^3	5.5×10^2	6.1×10^2	3.7×10^2	2.7×10^2	2.5×10^5	1.5×10^3

TCM –Total count of microorganisms, Lbc. – lactobacilli, Entero. – fam. *Enterobacteriaceae*, Ent. – enterococci, TMRae – aerobic thermoresistant microorganisms, TMRan – anaerobic thermoresistant microorganisms, Psychro. m. – psychrothrophic microorganisms, mikromyc. – micromycetes (yeasts and moulds)

it can be easy to see that the best results were achieved at the farm no. 1.

In goat milk, the highest average values of TCM were detected at the farm no. 3 using an environmentally friendly method. For sheep's milk, no significant difference was found out between the farms using

conventional or organic method.

The recommended values based on CSN 570529 related to cow's milk can be used for the evaluation of other monitored microorganism groups also proving technological importance. The number of psychrotrophic microorganisms should be up to 50000 in 1 mL,

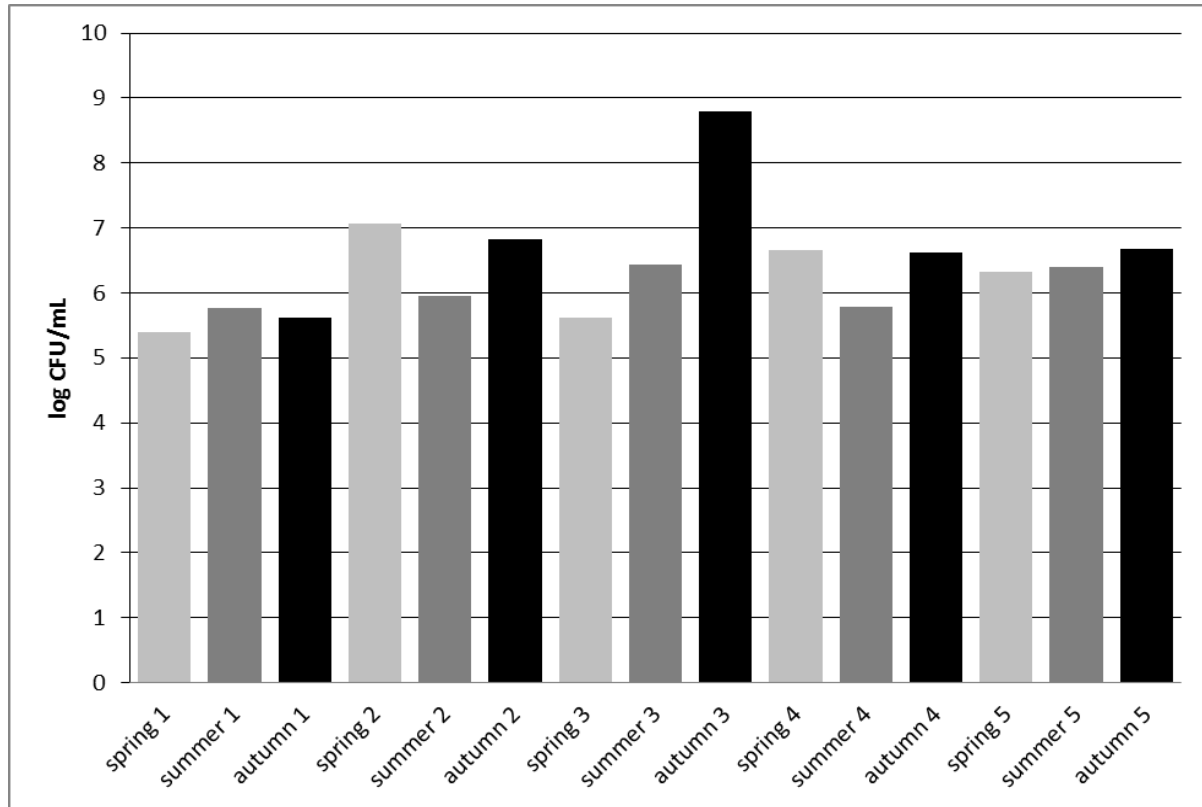


Figure 1 The average value of TCM in each seasons (2013 - 2014) for farms 1-5.

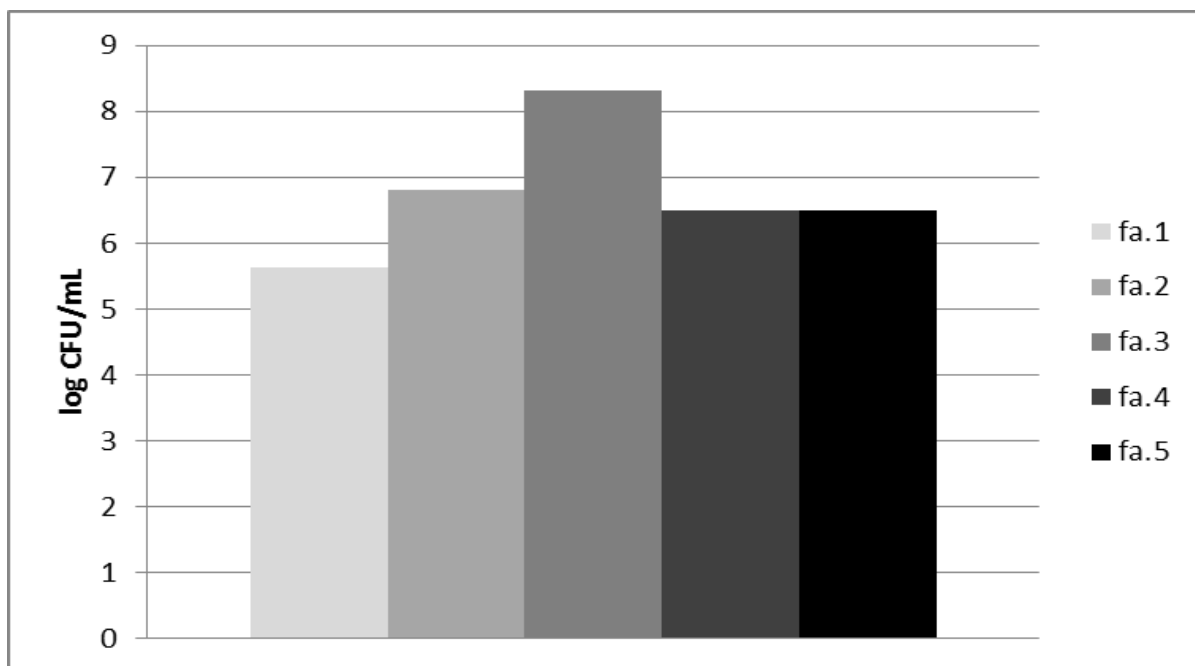


Figure 2 Average TCM the whole monitored period (2013 - 2014) for farms 1-5.

heat-resistant microorganisms up to 2000 in 1 mL, the highest number of coliforms up to 1000 in 1 mL, spore anaerobic bacteria should be negative in 0.1 mL. These microbiological limits are not determined for milk of small ruminants. In goat and sheep milk, the numbers are expected proportionately higher. Our identified microbial counts are corresponding with the findings (Tab. 3). High numbers TCM and coliform bacteria also mentioned **Oliveira et al. (2011)** and **Morgan et al. (2003)**.

The numbers of enterococci, lactobacilli but also coliform bacteria, yeasts and TCM samples of raw goat milk are lower up to several orders which are presented in the study by **Foschino et al. (2002)**, where significant differences are indicated between the farms.

High numbers of microorganisms in milk can cause spoilage unless the milk is quickly and efficiently cooled and subsequently pasteurized before further processing. In pasteurized milk, where the majority of contaminating microflora is destroyed, present microbial enzymes remaining active can cause the spoilage except to surviving microorganisms. The surviving microorganisms or microbial enzymes are also active and can spoil dairy products, which are made from such milk.

The results of microbiological analyzes show that the number of microorganisms in milk has been extensively individual and changeable. The final microbiological quality of the milk is caused by a number of factors (**Hejlová, 1997; Kalantzopoulos, 2003; Morgan et al., 2003; Malá et al., 2010; Cempírková et al., 2012**). The stage of lactation (Tab. 3, Fig. 1) did not prove a significant effect on the number of microorganisms in goat and sheep milk opposite to the number of somatic cells corresponding to the data of **Foschino et al. (2002)** and **Švejcárová et al. (2010)**. High numbers of microorganisms in goat and sheep milk may be primarily caused by the insufficient cleaning and sanitizing of milking equipment or low hygiene of hand milking. An important role may also act the cooling rate of milk and purity of cooling equipment. The method for cooling milk (cooling equipment versus the use of special glasses, see Tab. 2) may play an important role, especially in the farms from 1 to 3 producing goat milk.

CONCLUSION

From the above mentioned results, the following conclusions can be suggested. The bacterial counts of raw goat and sheep milk are highly variable and influenced by a number of important factors in the course of lactation and year. The bacterial numbers are not affected by the stage of lactation. Due to the small sample size and short term of monitoring, it can not be clearly said which farming method is preferable in the terms of microbial milk contamination. More important role acts the access, care and responsibility of the farmer apart from the mentioned factors. Not only for this reason, the microbiological analysis should be repeated for the following periods.

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