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Changes in the somatic cells counts and total bacterial counts in raw goat milk during lactation and their relationships to selected milk traits

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ABSTRACT

The main aim of our study was to assess changes in the somatic cell count (SCC) and total bacterial count (TBC) in the raw milk of White Short-haired (WSH) goats ($n = 13$) during lactation and their relationships to daily milk yield (DMY), basic milk components, the acidity of milk and California mastitis test (CMT). All monitored goats were in the second lactation and throughout the study, all these goats were clinically healthy. Individual milk recording and sampling of each goat during lactation were carried, from the end of April to October on the mean 54, 89, 124, 159, 194 and 229 day of lactation and all monitored goats were milked by hand. Throughout lactation, the mean values of Log SCC ranged from 5.60 to 5.91 and the mean values of Log TBC from 2.85 to 3.58, whilst the SL had no significant effect on any of these traits. The Log SCC had a significant positive correlation ($p \leq .01$) only with CMT, which suggests that CMT may be a good predictor of the SCC. On the other hand, all correlations of Log TBC with all other traits were insignificant. Regarding the effect of the SL on all other monitored traits, it had a highly significant effect ($p \leq .01$) on the DMY, contents of fat and total protein and titratable acidity. The SL had also a significant effect ($p \leq .05$) on the pH. Nevertheless, the SL had no significant effect on lactose content and CMT. The results of our study suggest that in clinically healthy WSH goats, the SCCs in goat milk after milking should only rarely be higher than 1,000,000 cells/mL and if strict milking hygiene is applied, the TBCs should be significantly lower than 500,000 cfu/mL. The present study also suggests that the SCCs in milk in WSH goats are comparable to their counts in most other breeds.

HIGHLIGHTS

- The stage of lactation had no significant effect on the SCC and TBC.
- In clinically healthy WSH goats, the SCCs in raw milk should rarely be higher than 1,000,000 cells/mL.
- When strict milking hygiene is applied in clinically healthy goats, the TBCs in milk after milking should be significantly lower than 500,000 cfu/mL.

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Introduction

The milk yield and composition of goat milk are affected by a number of different factors. The most important factors affecting both the quantity and quality of goat milk are management practices, production systems, breeding and genetics, health and nutrition (Goetsch et al. 2011; Pajor et al. 2019), whilst the essential quality traits of this milk are also the somatic cell count (SCC) and total bacterial count (TBC). Many studies show that the SCCs in goat milk compared to their counts in cow milk, due to the apocrine milk secretion process in goats, are generally higher and show higher variability. The SCC also represents a sensitive marker for udder health and is considered to be a useful parameter to evaluate the relationship between intramammary infection (IMI) and changes in milk characteristics (Raynal-Ljutovac et al. 2007). A large number of non-infectious factors can significantly affect the SCC in goat milk, whilst the most important of them are a fraction of milking, the

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time between milking, stage of lactation, number of lactation and breed (Jiménez-Granado et al. 2014). According to Paape et al. (2001), the SCCs in clinically healthy goats range from 270,000 to 2,000,000/mL and Raynal-Ljutovac et al. (2005) state that the cell count threshold established for goat milk for payment system in France, as in other countries such as the USA, is 1,000,000 cells/mL. In addition, Souza et al. (2012) published that most milk SCC thresholds used to identify IMIs range from 500,000 to 1,000,000 cells/mL. However, in the EU there is no legal limit for the SCC in goat milk.

The TBC is commonly used to evaluate the bacteriological quality of bulk milk. Many studies show, that the bacteriological quality of goat's milk is mainly influenced by the milking method, water quality, hygiene of milking equipment and storage tanks, hygiene of milkers, the environment in the milking parlour and hygiene of transport. However, Contreras et al. (2003) state that a high prevalence of IMI also contributes to an increase in bacterial counts. Ying et al. (2002), Delgado-Pertiñez et al. (2003) and Ramsahoi et al. (2011) published, that the TBCs in bulk samples range from 10^3 to 10^6 Colony Forming Units (cfu)/mL. Apart from the SCC, the EU determines for small ruminants milk the limits for the TBC in bulk milk when these limits are: 1500×10^3 cfu/mL in milk subjected to heat treatment and 500×10^3 cfu/mL for milk not subjected to heat treatment. However, in our opinion, the basic condition for low values of the TBC in bulk milk is their low counts after milking, because during the subsequent handling of milk the values of this trait are mainly affected by the level of hygiene and cooling rate of milk to the optimal temperature. Therefore, the main aim of the present study was to evaluate changes in SCC and TBC in the raw milk of White short-haired goats during lactation and their relationships to daily milk yield (DMY), basic milk components, the acidity of milk and California mastitis test (CMT) score. An integral part of our study was to assess the effect of the stage of lactation (SL) on the DMY, basic milk components, acidity of milk and CMT.

Material and methods

Animals, nutrition and experimental design

The study was carried out on a special goat dairy farm located in the northern part of the South Moravian Region of the Czech Republic (an altitude of 575 m above sea level; an average annual temperature of 6.5 °C; annual precipitation of 580 mm).

Thirteen goats of the White short-haired breed (WSH) in the second lactation were involved in the study, while this breed is currently included among European animal genetic resources. At the beginning of the study and during the whole lactation all monitored goats were clinically healthy. On the farm, a strict selection of goats for lower mastitis occurrence has been carried out for a long time and all goats were also under permanent veterinary supervision because the co-owner of the farm is a vet. The kidding occurred in the first half of March and the weaning of kids was carried out at their average age of 49 days. After weaning all monitored goats began to be machine milked twice a day. During lactation, the daily feed ration of goats consisted of haylage (1 kg/doe), meadow hay (0.5 kg/doe), concentrate mixture (1 kg/doe), pasture (*ad libitum*) and mineral lick (*ad libitum*). The composition of the concentrate mixture was as follows: triticale (40%), feeding barley (40%), oat (10%) and toasted soya (10%). During our study, all goats were kept in one flock under identical conditions without any discernible differences in their nutrition or management.

Milk recording and sampling

Individual milk recording and sampling of each goat were carried out from the end of April to October on the mean 54, 89, 124, 159, 194 and 229 days of lactation. As mentioned before, after weaning all monitored goats began to be machine milked twice a day. However, in the days when milk recording and sampling were carried out, all monitored goats were milked by hand. Milk yield recording was carried out in the morning milking (7 a.m.) and in the evening milking (7 p.m.), while milk samples were taken only from the morning milking. Individual milk samples were cooled to 5–8 °C and transported to the specialised milk laboratory at Mendel University in Brno and to the private Laboratory for Milk Analysis in Brno-Tuřany (Bohemian-Moravian Association of Breeders, a.s.). All monitored goats were dried off in the first decade of November.

Milk analysis

The SCC was determined using fluoroopto-electronic apparatus BENTLEY 2500 (Czech State Standard EN ISO No. 13366-2). The TBC was determined according to CSN EN ISO 4833-1 (560083). (Microbiology of the food chain – Horizontal method for the enumeration of microorganisms. Part 1: Colony count at 30 °C by

the pour plate technique). The DMY was recorded on the day of sampling as the total yield of the morning plus evening milking. Fat (F) content (in %) was determined by Gerber's acidobutyrometric method (Czech Technical Standard ISO No. 2446). Total protein (TP) content (in %) was determined according to the Czech Technical Standard EN ISO 8968-1 using a Kjeltec (Foss Electric, Denmark). Lactose (L) content (in %) was determined polarimetrically (Czech Technical Standard No. 570530). Active acidity (pH) was measured with the pH-meter WTW 95 with the probe WTW SenTix 97. Titratable acidity (TA, in °SH) was determined by titration using the Soxhlet–Henkel method (Czech State Standard No. 570530). The CMT was carried out using KerbaTEST (Albert Kerbl GmbH, Germany) and the CMT score was recorded as 1, 2, 3 and 4 (1: negative reaction, 2: traces, 3: weak positive reaction and 4: strong positive reaction).

Statistical analysis

The SCCs and TBCs were transformed into logarithmic forms to normalise their frequency distribution before performing statistical analysis. Statistical analyses were performed using the STATISTICA software, version 12. ANOVA analysis was used to study the differences in all monitored traits during lactation. To identify individual significant contrasts within the lactation stages, Sheffe's test was used. To asses the level of the correlation coefficient among all particular variables Pearson's correlation was carried out. The differences were considered significant if $p \leq .05$.

Results

The effect of the SL on the Log SCCs and Log TBC is presented in Table 1, the correlations of Log SCC and Log TBC with all other monitored traits are presented in Table 2. The SL had no significant effect on both Log SCC and Log TBC (Table 1). Throughout the lactation, the mean values of Log SCC ranged from 5.60 to 5.91 and the mean values of Log TBC from 2.85 to 3.58. The most noticeable, but insignificant increase in both Log SCC and Log TBC was found between the first and second sampling (in Log SCC from 5.60 to 5.91 and in Log TBC from 3.23 to 3.58). At the second and third sampling, the Log SCC values were relatively very balanced (5.91 and 5.87). However, between the third and fourth sampling, an insignificant decrease in the values of this trait was registered (from 5.87 to 5.72), but thereafter, until the end of lactation, a gradual increase in Log SCC from 5.72 to 5.83 was again found. Regarding the Log TBC, the values of this trait decreased insignificantly between the second and third sampling (from 3.58 to 3.03). However, subsequently, until the end of lactation, the Log TBCs were relatively very balanced and ranged from 2.85 to 3.17. The overall means of Log SCC and Log TBC for the entire lactation were 5.79 and 3.15. Throughout the lactation, the range of Log SCC values in individual samples was from 4.96 to 6.52 and in case of Log TBC from 0.70 to 4.58. The Log SCC had a significant positive correlation ($p \leq .01$) only with CMT (Table 2). On the other hand, all correlations of Log TBC with all other traits were insignificant. The correlation between Log SCC and Log TBC was also insignificant.

The effect of the SL on all other monitored traits is presented in the Table 3. The overall means of all other monitored traits for the whole lactation were as follows: DMY = 2.40; F = 3.30%; TP = 2.85%; L = 4.58%, pH = 6.69; TA = 6.33 °SH and CMT score = 2.12. Regarding the effect of the stage of lactation (SL) on all other monitored traits (Table 3), it had a highly significant effect ($p \leq .01$) on the DMY, contents of F and TP and TA. The SL had also a significant effect ($p \leq .05$) on the pH. On the other hand, the SL had no significant effect on the L content and CMT score. The highest DMY was found on the 89th day of lactation (3.28 L), while subsequently a gradual decrease of its value was recorded to the level of 1.35 L at the end of lactation. In the first half of lactation, the contents of F significantly decreased (from 3.43 to 2.78%) and the contents of TP differed slightly (from 2.60 to 2.71%). Whereas, in the second half of

Table 1. Mean values of SCC and TBC during lactation.

	Mean day of lactation						Range of individual samples	Overall mean	Significance	SE
Trait	54	89	124	159	194	229				
SCC	400×10^3	807×10^3	746×10^3	528×10^3	649×10^3	683×10^3	92×10^3 to 3336×10^3	636×10^3	n.s.	129.17
Log SCC	5.60	5.91	5.87	5.72	5.81	5.83	4.96–6.52	5.79	n.s.	0.04
TBC, cfu/mL	1.7×10^3	3.8×10^3	1.1×10^3	7.1×10^2	1.5×10^3	1.1×10^3	0.5×10^1 to 3.8×10^4	1.7×10^3	n.s.	520.79
Log TBC	3.23	3.58	3.03	2.85	3.17	3.05	0.70–4.58	3.15	n.s.	0.08

n.s.$p > .05$.
n.s.: not significant; Log: logarithm; SCC: somatic cell count; SE: standard error; TBC: total bacterial count.

Table 3. Mean values of DMY, basic milk components, acidity and CMT during lactation.

Trait	Mean day of lactation						Range of individual samples	Overall mean	Significance	SE
	54	89	124	159	194	229				
Daily milk yield, L	2.65^{ABC}	3.28^{ADEFG}	2.84^{DHI}	2.28^{EJ}	2.02^{BFHK}	1.35^{CGIJK}	0.60–4.80	2.40	**	0.11
Fat, %	3.43^{A}	3.19^{bC}	2.78^{AbdEF}	3.04^{dG}	3.35^{EH}	4.00^{CFGH}	2.06–5.23	3.30	**	0.08
Total protein, %	2.60^{AB}	2.71^{CD}	2.68^{EF}	2.70^{GH}	3.05^{ACEGI}	3.37^{BDFHI}	2.18–4.03	2.85	**	0.04
Lactose, %	4.69	4.60	4.54	4.40	4.64	4.60	4.00–5.22	4.58	n.s.	0.03
pH	6.68^{A}	6.68^{B}	6.77^{ABCDE}	6.68^{C}	6.68^{D}	6.65^{E}	6.52–6.98	6.69	*	0.01
Titratable acidity, °SH	5.41^{ABC}	5.67^{dEF}	5.95^{GH}	6.34^{AdIJ}	7.06^{BEGI}	7.54^{CFHJ}	4.37–9.42	6.33	**	0.12
California mastitis test	1.77	2.31	1.92	2.15	2.08	2.46	1–3	2.12	n.s.	0.07

n.s.$p > .05$; *$p < .05$; **$p < .01$. The effect of stage of lactation on the monitored traits (tested by one-way ANOVA).
DMY: daily milk yield; CMT: California mastitis test; SE: standard error; L: litre; oSH: degree Soxhlet-Henkel.
$^{a–d}$The same characters on one line indicate the statistically significant difference between individual samples (tested by paired Student's *t*-test) $p < .05$; $^{A–K}$the same characters on one line indicate the statistically significant difference between individual samples (tested by paired Student's *t*-test) $p < .01$.
n.s.: not significant; SE: standard error.
California mastitis test score was recorded as 1, 2, 3 and 4 (1: negative reaction, 2: traces, 3: weak positive reaction and 4: strong positive reaction).

lactation the contents of both of these milk components gradually increased. The pH of milk was relatively very balanced during the whole lactation (from 6.65 to 6.68), except for the third sampling when the pH increased to the value of 6.77. By contrast, the TA gradually increased throughout the lactation from 5.41 °SH to 7.54 °SH. The contents of L declined slightly between the first and fourth sampling (from 4.69 to 4.40%), whilst in the fifth and sixth sampling the values of this trait increased slightly to 4.64% and 4.60%. The CMT score was relatively very balanced during lactation and ranged from 1.77 to 2.31.

Discussion

The effect of the stage of lactation on the SCCs and the relationships of the SCC with all monitored traits

All goats in our study were under permanent veterinary supervision and during the whole lactation, no signs of clinical mastitis and no changes in the appearance of milk were observed in these goats. Strict selection of goats for lower mastitis occurrence has also been applied in the monitored flock for a long time. These facts were reflected in relatively low mean values of Log SCC (Table 1), while their values were slightly higher than reported earlier Kuchtík et al. (2015) in Brown short-haired breed and Margatho et al. (2018) in Serrana goats and comparable with results published by Ying et al. (2002) in Alpine goats, Vacca et al. (2010) in Sarda goats and Albenzio et al. (2016) in the Garganica breed. All mean values of SCC in the present study were also lower than threshold values published by Raynal-Ljutovac et al. (2005) and Skeie (2014). On the other hand, Delgado-Pertiñez et al. (2003) and Sramek et al. (2018) found higher SCCs in Alpine goats as compared to their values in the present study. Vacca et al. (2010) and Albenzio et al. (2016) state, that SCCs in goat milk increase as lactation advances. The highest increase in the Log SCC value during lactation in the present study was found between the first and second sampling. In our opinion, this increase was affected mainly by a considerable temperature drop on the pasture which began a few days before the second sampling and not by the IMI, because no clinical signs of mastitis were observed in all goats in this period. It is surprising that the considerable drop in the environmental temperature did not affect also the DMY, which was the highest for this period within the entire lactation.

Goat milk with an SCC higher than 3,500,000/mL should not be accepted for marketing because such milk displays a high risk of pathogens and toxins occurrences and this milk has also poor industrial quality (Silanikove et al. 2010). Persson and Olofsson (2011) using deLaval cell counter found in infected goat mammary glands the mean SCC value of 711,000 cells/mL However, according to Paape et al. (2001), the SCC value in healthy goats should not exceed 1,000,000 cells/mL for individual goats. In the present study, a total of 78 individual milk samples (IMS) were analysed during lactation. In 46 of IMS, (i.e. 59%) the SCCs were lower than 500,000/mL and in 21 of IMS, (i.e. 27%) their counts ranged from 501,000 to 1,000,000 cells/mL. In contrast, only in 11 of IMS, (i.e. 14%) were SCCs higher than 1,000,000/mL, while this limit was exceeded once in the first sampling, three times in the second and fifth sampling and four times in the sixth sampling. With regard to individual goats, only in one of the monitored goats, the SCC was twice higher than 1,000,000/mL during the whole lactation. However, in all of these goats, typical mastitis symptoms were not shown, and all these goats also displayed relatively low total bacterial counts (TBCs), which ranged from 59 to 38,400 cfu/mL. In addition, the SCCs over 1,000,000/mL did not significantly

influence all other assessed traits. Regarding the SCCs in IMS in the present study, it seems that their higher levels were affected mainly by the daily SCC variability which is in line with the finding of Zeng et al. (1997) and Haenlein (2002), when Raynal-Ljutovac et al. (2007) state that physiological factors in goats may account for up to 90% of variation in SCC, and can be responsible in variations greater than 1×10^6 cells/mL. The assessment of relationships between the Log SCC and all other monitored traits (Table 2) showed that this trait had only a positive significant correlation ($p \leq .01$) with CMT, while Haenlein (2002) also found between these two traits the same correlation. However, in our opinion, this positive correlation was not affected by IMI, because all other assessed correlations with the Log SCC were found to be insignificant.

The effect of the stage of lactation on the TBCs and the relationships of the TBC with all monitored traits

All mean values of Log TBC and all values of TBC in IMS were relatively very low during the lactation (Table 1) and the SL had no significant effect on Log TBC which is in line with Ying et al. (2002); Foschino et al. (2002) and Delgado-Pertiñez et al. (2003). In contrast Zeng and Escobar (1995); Zweifel et al. (2005) and Vacca et al. (2018) state, that the SL has a significant effect on the TBC values. The study conducted by Zeng and Escobar (1995) also showed that the increase of TBC values may be related to the decline of milk production in late lactation. In addition, Zweifel et al. (2005) state, that the increase of TBC can be affected by an increase in environmental temperature. However, in our study, the insignificantly highest Log TBC was found at the peak of lactation, respectively in early June, when a considerable drop in environmental temperature was recorded in this period. In this context, it should also be added that the mean highest Log TBC was 3.58, which means that TBC was very low (3.8×10^3 cfu/mL). Based on our results, it can be stated that if milking hygiene is consistently high and goats are in good health (without IMI), the SL and varying changes in environmental temperature should not affect considerably the values of Log TBC. The mean values of Log TBC in the present study were significantly lower than the determined limit by EU for TBC and the value found by Vacca et al. (2010), while this study was based also on the evaluation of IMS. In contrast, Margatho et al. (2018) who also based evaluation on IMS found lower values for TBC.

As for TBC in bulk milk, studies carried out by Foschino et al. (2002) and Ramsahoi et al. (2011) show that its value is significantly higher in bulk milk than in IMS. Also, D'Amico and Donnelly (2010) who performed the evaluation on five goat farms found relatively very high mean values of Log SPC (higher than 4.2) in the four of these farms. However, in one farm they found a very low mean value of Log SPC (1.2), while this value is significantly lower than those found in our study. Very low values of logarithmic bacterial counts in goat bulk milk (from 1.59 to 1.81) were also reported by Vacca et al. (2018). Regarding the comparison of TBCs in bulk milk and in IMS, it should be also emphasised that the TBCs in bulk milk are generally higher compared to IMS because their counts are also affected by other factors such as for example the level of sanitation of pipelines and storage tanks, milk cooling rate and temperature of milk in storage tanks.

As for correlations between the LogTBC and all other individual assessed traits in the present study, all these correlations were found to be insignificant.

Recently, quite a lot of studies have dealt also with relationships between SCC and TBC. Whereas, the finding of these studies is full of contradictions. Foschino et al. (2002), Delgado-Pertiñez et al. (2003) and Vacca et al. (2010) found this correlation to be insignificant, which is in line with the finding of our study. In contrast, Zeng and Escobar (1995) and Koop et al. (2009) found this correlation to be significantly positive. In our opinion, increasing of SCCs in the milk of healthy goats may not have a significant effect on TBCs and vice versa, since the SCCs in healthy goats are primarily affected by the stage of lactation, whereas, the TBCs are mainly affected by milking hygiene.

The effect of the stage of lactation on the DMY, basic milk components, acidity of milk and CMT score

Many studies showed that the DMY decreases and the F and TP contents gradually increase after the peak of lactation. The same trends were also found in the present study (Table 3), whilst the SL had a significant effect ($p \leq .01$) on all aforementioned traits, which is in line with Králíčková et al. (2013) and Albenzio et al. (2016). In contrast, Salari et al. (2016) did not find a significant effect of the SL on both fat and protein content.

Besides that, many studies showed that the pH of milk is affected by mastitis and according to Pirisi et al. (2007) its value affects milk aptitude for rennet coagulation and cheese-making. Moreover, also

titratable acidity (TA) plays a fundamental role in coagulation (De Marchi et al. 2009), while its values are considerably affected by the protein content of milk (Strzalkowska et al. 2009), Optimal values for the pH and TA of raw goat milk range from 6.4 to 6.8 and from 6.2 to 7.8 °SH, respectively. In the present study, the SL had a significant effect on both pH and TA. However, the pH values, with the exception of the third sampling, were relatively very balanced and its one-time increase in the third sampling did not have a significant effect on any of the monitored traits. The pH values in the present study were also comparable both with their optimal range and with their values reported by Vacca et al. (2018) and Currò et al. (2020). Compared to the values of pH, the values of TA gradually increased during lactation which is in line with Kuchtík et al. (2015). However, in the first half of lactation, the TA values were slightly lower than the aforementioned optimal range, but this fact did not affect any of the monitored traits. In conclusion to the evaluation of TA, it should be added that between the TA and TP content was found a positive correlation ($p \leq .01$), which suggests, similar to Strzalkowska et al. (2009), that the higher TP content, the higher TA.

As mentioned before, the SL had no significant effect on CMT. On the other hand, a strong positive correlation ($p \leq .01$) was found between the CMT and Log SCC. This finding is consistent with the conclusion reported by Persson and Olofsson (2011) and suggests that the CMT may be a good predictor of the SCC. However, since no mastitis was detected in any of the monitored goats, it is not possible to state whether the CMT is suitable or unsuitable for the diagnosis of this serious disease.

Conclusions

In the present study, the stage of lactation had no significant effect on both SCC and TBC, and the correlation between these traits was also insignificant. However, the stage of lactation had a significant effect on the daily milk yield, contents of fat and total protein, titratable acidity, and pH. The results of the present study suggest that the somatic cell counts and especially total bacterial counts in raw goat milk after milking are at relatively low levels when is applied strict selection against mastitis, regular health check, and strict adherence to milking hygiene. The results of the present study also suggest that the somatic cell counts in milk in White short-haired goats are comparable to their counts in most other breeds and that the California mastitis test may be a good predictor of the somatic cell count.

Ethical approval

Experimental procedures and animal care conditions followed the recommendation of European Union directive 86/609/EEC and were approved by Expert Commission for Ensuring the Welfare of Experimental Animals of Mendel University in Brno.

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