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EFFECT OF PARITY AND STAGE OF LACTATION ON MILK YIELD, COMPOSITION AND QUALITY OF ORGANIC SHEEP MILK

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Abstract

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Effect of parity (PA), stage of lactation (SL) and interaction of PA x SL on daily milk yield (DMY), composition (contents of total solids, fat, total protein and lactose) and quality (somatic cell and bacteria counts) of organic sheep milk were evaluated using 20 ewes of East Friesian which were reared on an organic farm in Habří. Ewes were on the 2nd (n = 10) and 3rd (n = 10) lactation. During the experiment ewes were reared on permanent pasture. The milk records and samplings were carried out on average 75th, 132th and 190th day of lactation. All parameters were determined by standard methods. The PA had a significant effect only on somatic cell count (SCC). On the other hand, SL had a significant effect on daily milk yield (DMY), contents of total solids (TS), total protein (TP) and lactose (L). The SL also had a significant effect on total bacteria count (TBC), psychrotrophic bacteria count (PBC), total coliform count (TCC) and SCC. The interaction of the PA x SL had a significant effect on DMY, TS and TP contents and PBC. Positive and significant correlations were found among the SL and contents of TS and TP. Significant and positive correlation was observed between the PA and SCC, but on the other hand, significant and negative correlation was found between the SL and SCC.

organic milk, sheep, composition of milk, somatic cells, microorganisms

In the Czech Republic (CR) the sheep milk production is still a minor, however during last year was reported an increase in the numbers of dairy sheep whilst presently is reared more than 1 500 milking ewes. Sheep milk has a high nutritional value, contains about 200 active substances (Horák *et al.*, 2004) and is specific to markedly higher contents of total solids, fat, total protein, casein, whey protein and ash compared to cow's and goat's milk (Bencini and Pulina, 1997). Due to this, sheep milk is an excellent raw material especially for cheese production. In the CR, all milk is processed into above all cheese directly on the farms. Composition of sheep milk and its production are influenced by a number of factors while the parity and stage of lactation being among the most important (Oravcová *et al.*, 2007). The effect of the parity and stage of lactation on milk composition in different sheep breeds were studied by Sevi *et al.* (2000), Aganga *et al.* (2002), Nuda *et al.* (2003), Oravcová *et al.* (2007), Kuchtík *et al.* (2008), Novotná *et al.* (2009) and Konečná *et al.* (2011).

The primary prerequisite for making high quality milk product is the production of high quality raw milk by dairy farms. Milk can be easily spoilt if the animals are given unsuitable feed or are handled in the wrong way before and during milking, or if the milk is handled incorrectly after milking, including the process of cooling (Jandal, 1996). The composition of microflora of milk can be very varied, because, as stated by Nester *et al.* (1998), milk is an excellent growth medium for many microorganisms. The milk in the mammary gland of healthy animals is almost sterile (Cempírková *et al.*, 1997), but during and after milking the origin microflora is markedly changed due to secondary contamination. Sources of secondary contamination are most often the surface of the mammary gland, dust, water, feedstuff, litter and milking equipment (Smetana, 2009). Currently, closed milking system, better sanitary design of equipment, more efficient cleaning of udder and teats enable to produce raw milk with extremely low microbial contamination (Barbano *et al.*, 2006).

Microbiological quality of raw milk is assessed in large extend by total bacteria count (TBC) and somatic cell count (SCC), and these parameters are routinely measured and compared. Microbial analysis and SCC have been used to diagnose mastitis in ewes. It was found that *Staphylococcus aureus* (together with *Streptococcus agalactiae* and *Streptococcus uberis*) persists as the main pathogen causing mastitis (Hariharan *et al.*, 2004 and Vyletělová *et al.*, 2010). According to Leitner *et al.* (2004), mastitis causes the changes in SCC and milk composition, while lactose content is lower, whey protein and albumin contents are significantly higher. As reported by Gonzalo *et al.* (2010), SCC and TBC are important to the farmers, cheese manufacturers, and consumers because they are the major factors in determining safety and hygienic quality of the final products. Thus, the European Union establishes the limits for TBC (Council Regulation EEC 853/2004) in ovine milk. According to above mentioned regulation, the maximal limit of TBC in raw milk is $1.5.10^6$ CFU.ml^{-1}. In the Czech Republic does not yet exist the standard for determining the limit of SCC in small ruminants, but e.g. Bianchi *et al.* (2004) reported the limit for subclinical mastitis at 500 000 cells.ml^{-1}.

As mentioned by Hantsis-Zacharov and Halpern (2007) and Kumaresan *et al.* (2007), controlling the content of psychrotrophic bacteria is also very important, because these Gram-negative bacteria are able to produce heat-stable extracellular proteases, as well as lipases, that are potentially harmful for technological quality of milk and milk products. Proteases are associated with bitterness in milk and reduces yield of soft cheeses and lipases cause flavour defects in cheeses and other milk products. In Europe, psychrotrophic bacteria count (PBC) of raw milk should not exceed 50.10^3 CFU.ml^{-1} (Cempírková and Mikulová, 2009). Furthermore, according to Vyletělová and Hanuš (2000) and Cempírková and Mikulová (2009), lipolytic and proteolytic activities, supported by psychrotrophic bacteria, are considered insignificant at PBC lower than 10^6 CFU.ml^{-1}.

The sheep milk quality is determined not only by its nutrient content but also by its hygienic aspects. Therefore, the objective of this study was to evaluate the effect of the parity and stage of lactation on milk yield, composition (TS, F, TP and L) and quality (SCC, TBC, PBC and TCC) of organic sheep milk.

MATERIALS AND METHODS

Evaluation of effect of parity (PA), stage of lactation (SL) and interaction of PA x SL on daily milk yield, composition and quality (somatic cell and bacteria counts) of organic sheep milk were evaluated using 20 ewes of East Friesian breed which were reared on an organic farm in Habří. The farm is situated at an altitude of 430m above sea level with an average annual temperature of 6.9 °C and annual precipitation of 865mm. Samples were obtained from 10 ewes on the second lactation (PA2) and from 10 ewes on the third lactation (PA3). Individual milk records and samplings were carried out three times in the period from May to September 2009 (on the average 75th, 132th and 190th day of lactation). During the experiment, the daily feed ration of ewes consisted of permanent pasture (*ad libitum*), organic mineral lick (*ad libitum*) and organic oat (0.5kg/ewe/day). The milking of ewes was carried out, during the experiment, once a day (8 am). During the experiment, all of the ewes were reared in one flock under identical conditions without any discernible differences in nutrition or management.

Individual milk samples were cooled to 5–8 °C and transported in a thermo-box to a specialized 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.). As part of the laboratory analysis, the following determinations of milk composition and quality were carried out: total solids (TS), fat (F), total protein (TP) and lactose (L) contents, numbers of total bacteria, psychrotrophs and total coliforms. The evaluation of somatic cell count (SCC) and daily milk yield (DMY) was also an integral part of this study.

TS content (%) was determined gravimetrically; by over drying at 102 °C to constant weight (Czech technical Standard ISO No. 6731, 1998). Fat content (%) was determined by Gerber's acidobutyrometric method (Czech Technical Standard ISO No. 2446, 2010). TP content (%) was determined using a PRO-MILK apparatus (manufactured by the Danish Co. Foss Electric; Czech Technical Standard No. 570530, 1974). L content (%) was determined polarimetrically (Czech Technical Standard No. 570530, 1974). SCC was determined using fluoro-opto-electronic apparatus BENTLEY 2500 (Czech Technical Standard EN ISO No. 13366-2, 2007). Samples for microbiological determination were milked by hand and were collected in sterile plastic containers. Before sampling the milker washed his hands carefully. Numbers of total bacteria, psychrotrophs and total coliforms were determined by standard plate count method. Milk samples were diluted by the decimal dilution (9 ml of distilled water and 1ml of milk sample). Total bacteria count (TBC) was determined on plate count agar with skimmed milk (PCA, Biokar Diagnostic, France); inoculated plates were incubated at 30 °C for 72 hours (Czech Technical Standard EN ISO No. 4833, 2003). Samples for the determination of psychrotrophic bacteria count (PBC) were spread also on PCA with skimmed milk (Biokar Diagnostic; France); plates were incubated at 6.5 °C for 10 days according to the Czech Technical Standard ISO No. 6730, 2007. Total coliform count (TCC) was monitored in process of cultivation on VRBL agar (Biokar Diagnostic; France) at 37 °C for 24 hours (Czech Technical Standard ISO No. 5541-1, 1996). The colonies of microorganisms which grew out in Petri dishes were counted after termination of cultivation, while the number of these colonies were recalculated using the following mathematical formula. The final bacterial counts are expressed in specific units CFU (Colony Forming Units) per 1 ml of milk sample.

$$N = \frac{\Sigma C}{V \times d \times (n_1 + 0{,}1 \times n_2)},$$

where:

N ...... number of CFU in 1 ml of milk sample
ΣC .... the sum of colonies grown in Petri dishes at two consecutive dilutions
V .... volume of inoculum
d .... first dilution factor used to calculate the dilution
n_1...... number of Petri dishes used for the calculation of the first dilution
n_2...... number of Petri dishes used for the calculation of the second dilution.

Recorded data were statistically analyzed using the least-squares method. The systematic effects were PA (two classes), the SL (three classes) and the interaction of PA x SL. Statistical analysis was carried out using the mathematical-statistical package STATISTICA version 9.0.

RESULTS AND DISCUSSION

Effect of parity (PA), stage of lactation (SL) and the interaction of PA x SL on daily milk yield and basic milk composition (contents of total solids, fat, total protein and lactose) is presented in Table I.

The PA had no significant effect on daily milk yield (DMY), which is in line with the results published by Pokorná *et al.* (2010), but on the other hand, Oravcová *et al.* (2006) found a significant

I: *L.S.M. and S.E.M values of daily milk yield and particular components of organic sheep milk in parities 2nd and 3rd*

Parameter	ADL	n	Parities				Level of significance		
			PA2		PA3		PA	SL	SL x PA
			L.S.M.	S.E.M.	L.S.M.	S.E.M.			
DMY			**		**		NS	**	**
	75.	10	0.72^{A}	0.11	0.83^{A}	0.32			
	132.	10	0.80^{A}	0.25	0.66^{A}	0.20			
	190.	10	1.57^{B}	0.35	1.40^{B}	0.47			
	Mean		1.03	0.45	0.97	0.46			
TS					*		NS	*	*
	75.	10	17.82	0.91	17.39^{a}	1.16			
	132.	10	17.30	0.66	17.75^{ab}	0.83			
	190.	10	18.14	0.72	18.56^{b}	0.86			
	Mean		17.75	0.82	17.90	1.05			
F			*				NS	NS	NS
	75.	10	7.12^{a}	0.95	6.75	0.99			
	132.	10	6.28^{b}	0.54	6.84	0.76			
	190.	10	6.24^{b}	0.71	6.69	0.79			
	Mean		6.55	0.84	6.76	0.82			
TP			**		**		NS	**	**
	75.	10	4.73^{A}	0.17	4.65^{A}	0.21			
	132.	10	5.22^{B}	0.29	5.15^{B}	0.27			
	190.	10	5.94^{C}	0.23	5.96^{C}	0.17			
	Mean		5.30	0.55	5.25	0.58			
L					*		NS	*	NS
	75.	10	5.06	0.18	5.09^{a}	0.18			
	132.	10	4.90	0.16	4.86^{b}	0.14			
	190.	10	5.06	0.16	5.01^{ab}	0.15			
	Mean		5.01	0.18	4.99	0.18			

ADL = average day of lactation; SL = stage of lactation; n = number of cases; PA = parity; Mean = average value of lactation; DMY = daily milk yield (kg); TS = total solids content (%); F = fat content (%); TP = total protein content (%); L = lactose content (%); ABC values in the same columns were significantly different ($P \leq 0.01$); abc values in the same columns were significantly different ($P \leq 0.05$); ** = statistically highly significant ($P \leq 0.01$); * = statistically significant ($P \leq 0.05$); NS = statistically non-significant ($P > 0.05$)

effect of this factor on DMY. Furthermore, the PA had no significant effect on contents of total solids (TS), fat (F), total protein (TP) and lactose (L), which correspond to the results published by Pokorná *et al.* (2010). On the contrary, Sevi *et al.* (2000) found a significant effect of this factor on contents of all milk components under their study; also Konečná *et al.* (2011) reported a significant effect of the PA on contents of total solids (TS), fat (F) and total protein (TP). However, Marìa and Gabiña (1993) stated that the PA affects only contents of TS not F. The average DMY and lactose (L) content were during the whole lactation period slightly lower for the third lactation (PA3) compared with the second (PA2) lactation, which is consistent with the results published by Ploumi *et al.* (1998), Novotná *et al.* (2009) and Pokorná *et al.* (2010). The average value of content of TP was slightly higher on the PA2 in our experiment, however on the other hand, Sevi *et al.* (2000) and Oravcová *et al.* (2007) reported higher average TP content on the PA3. With regard to the average contents of TS and F, these were higher on the PA3, which is in agreement with the results of study carried out by Sevi *et al.* (2000). On the other hand, Konečná *et al.* (2011) reported the opposite trend.

The SL had a significant effect on DMY, which is consistent with the results published by Ochoa-Cordero *et al.* (2002). Also, the contents of TS, TP and L were affected by the SL. The same conclusions also report Bencini and Pulina (1997), Sevi *et al.* (2000; 2004) and Pokorná *et al.* (2010). On the other hand, F content was not affected by this factor. DMY of ewes was relatively stable between the 75th and 132nd day of lactation (from 0.66 to 0.83 kg), but on the 190th day of lactation the DMY greatly increased in both lactations (1.57 and 1.40 kg). In contrast, Nuda *et al.* (2003) and Kuchtík *et al.* (2008) reported the opposite trend. In our opinion, the low DMY till the 132nd day of lactation was probably caused by the inadequate production of green mass on pasture as a result of low rainfall from May to July. Ploumi *et al.* (1998) also point to the fact that long-term droughts and high temperatures lead to a drop in milk yield. Contents of TS and TP increased with advancing lactation, the same trend of L.S.M. values of TS and TP published Sevi *et al.* (2000; 2004) and Jaramillo *et al.* (2008) in their experiments. On the contrary, F content in our study gradually decreased during the lactation period, which is not in line with the results published by Aganga *et al.* (2002), Sevi *et al.* (2004), Sahan *et al.* (2005) and Novotná *et al.* (2009). The content of L was relatively well balanced during the whole observation; this conclusion corresponds with results published by Pugliese *et al.* (2000), Kuchtík *et al.* (2008) and Novotná *et al.* (2009). According to the Table I, we can see that the milk protein was the most variable component in comparison with the other components of the milk. Finally, it is necessary to complete that the interaction of PA x SL had a significant effect on DMY, TS and TP contents; on the other hand this factor had no effect on F and L contents. By contrast, Sevi *et al.* (2000) did not find a significant effect of the interaction of PA x SL on any of the above mentioned parameters of sheep's milk.

The effect of the PA, SL and its interaction on somatic cell and bacteria counts is summarized in Table II.

The PA had a significant effect on somatic cell count (SCC), which is not consistent with findings of Sevi *et al.* (2000). On the other hand, as mentioned by Pugliese *et al.* (2000), SCC significantly increased from PA2 to the fourth lactation, which is in line with results of our experiment, while L.S.M values of SCC were significantly higher in ewes on the PA3 as compared to ewes on the PA2. SCC was significantly effected also by the SL, which corresponds to conclusions published by Sevi *et al.* (2004). However, Pugliese *et al.* (2000), Sevi *et al.* (2000) and Pokorná *et al.* (2009) did not find a significant effect of this factor on SCC during their study. As regards SCC in ewes on the PA3, L.S.M. values were between the 75th and 132nd day of lactation almost identical, however, the 190th day of lactation SCC markedly decreased. A similar trend between the 75th and 132nd day of lactation was reported by Paape *et al.* (2006). The interaction of PA x SL had no significant effect on SCC, which is in line with results mentioned by Sevi *et al.* (2000). The L.S.M. values of SCC ranged from 28.00 to 117.40 thousands.ml^{-1} during the whole lactation. As regards SCC, it is necessary to add that none of the ewes did not suffer from subclinical mastitis during the observation. By the way, Bianchi *et al.* (2004) reported limit for subclinical mastitis at 500 000 of somatic cell per 1ml of milk sample.

The PA had no significant effect on total bacteria count (TBC) and psychrotrophic bacteria count (PBC). On the contrary, the SL had a significant effect on TBC, which is consistent with results published by Micari *et al.* (2002). It should be pointed out that values of TBC founded by these authors are markedly higher than our data. Talevski *et al.* (2009) and Malá *et al.* (2010) also reported in their studies higher values of TBC using the same system of milking. Furthermore, the SL also significantly effected PBC which is in agreement with Sevi *et al.* (2000). In the Table II we can see the similar dynamics of TBC and PBC values during the lactation period, while between the 75th and 132nd day of lactation was found in both lactations a significant increase their numbers, however after that, on the 190th day of lactation TBC and PBC decreased. As regards evaluating of the interaction of PA x SL, this factor influenced only PBC. In relation to above mentioned should be added that L.S.M. values of TBC did not exceed 1.5.10^{6} CFU. ml^{-1}, which is maximal limit of TBC for raw sheep milk (Regulation of the European Parliament and Council No. 853/2004).

The PA had no significant effect on total coliform count (TCC). On the other hand, the SL had a significant effect on TCC, which corresponds with results published by Sevi *et al.* (2000; and 2004). Coliforms as important indicators of primary and

II: *L.S.M. and S.E.M values of somatic cell and bacteria counts of organic sheep milk in parities 2^{nd} and 3^{rd}*

Parameter	ADL	n	Parities				Level of significance		
			PA2		PA3		PA	SL	SL x PA
			L.S.M.	S.E.M.	L.S.M.	S.E.M.			
SCC			*		*		*	*	NS
	75.	10	76.20^{a}	26.97	114.30^{a}	72.90			
	132.	10	55.90^{ab}	43.73	117.40^{a}	143.52			
	190.	10	34.00^{b}	22.74	28.00^{b}	17.58			
	Mean		55.37	35.87	86.57	99.57			
TBC			*		**		NS	**	NS
	75.	10	42.70^{a}	26.49	49.70^{A}	43.26			
	132.	10	133.50^{b}	144.66	150.30^{B}	112.36			
	190.	10	41.20^{a}	65.86	31.50^{A}	27.45			
	Mean		72.47	99.93	77.17	86.93			
PBC					*		NS	**	*
	75.	10	6.10	5.74	5.50^{ab}	2.32			
	132.	10	15.90	24.31	45.50^{a}	61.04			
	190.	10	0.90	1.52	2.20^{b}	3.85			
	Mean		7.63	15.31	17.73	39.54			
TCC			*				NS	*	NS
	75.	10	0.00^{a}	0.00	0.00	0.00			
	132.	10	0.90^{b}	1.45	0.80	1.03			
	190.	10	0.00^{a}	0.00	0.50	1.27			
	Mean		0.30	0.92	0.43	0.97			

ADL = average day of lactation; SL = stage of lactation; n = number of cases; PA = parity; Mean = average value of lactation; SCC = somatic cell count (thousands.ml^{-1}); TBC = total bacteria count (CFU.ml^{-1}); PBC = psychrotrophic bacteria count (CFU.ml^{-1}); TCC = total coliform count (CFU.ml^{-1}); ABC values in the same columns were significantly different ($P \leq 0.01$); abc values in the same columns were significantly different ($P \leq 0.05$); ** = statistically highly significant ($P \leq 0.01$); * = statistically significant ($P \leq 0.05$); NS = statistically non-significant ($P > 0.05$)

III: *Pearson correlations among parity, stage of lactation and all researched parametrs of milk*

	DMY	TS	F	TP	L	SCC	TBC	PBC	TCC
PA	–0.07	0.08	0.13	–0.04	–0.05	0.21^{*}	0.03	0.17	0.07
SL	0.63^{**}	0.33^{**}	–0,23	0.92^{**}	–0.09	$–0.35^{**}$	–0.04	–0.06	0.11

PA = parity; SL = stage of lactation; DMY = daily milk yield; TS = total solids content; F = fat content; TP = total protein content; L = lactose content; SCC = somatic cell count; TBC = total bacteria count; PBC = psychrotrophic bacteria count; TCC = total coliform count; ** = statistically highly significant ($P \leq 0.01$); * = statistically significant ($P \leq 0.05$)

secondary contamination of raw milk (Görner and Valík, 2004) occurred in milk samples only sporadically, while 77% of all milk samples were negative for these bacteria. Under study, TCC was very-well balanced on both lactations. A similar trend was reported by Sevi *et al.* (2000). Our observed values of TCC were markedly lower as compared to those of Sevi *et al.* (2000) and Malá *et al.* (2010). This fact points to the observing good hygienic principles during milking. Finally, it is necessary to complete that the effect of the interaction of PA x SL was not reported, which is in line with the conclusion published by Sevi *et al.* (2000).

Table III shows correlations among PA, SL and DMY, basic milk components and quality parameters of sheep milk. As we can see, significant and positive correlation was found between the PA and SCC. This means that with increasing order of lactation also increased the number of somatic cells, which is consistent with results published by Pugliese *et al.* (2000). On the other hand, as regards correlation SL vs. SCC this was significant, but negative. On the contrary, Luengo *et al.* (2004) and Raynal-Ljutovac *et al.* (2006) published the opposite trends; however, Paape *et al.* (2006) consistently with our results reported a decrease of SCC with advanced stage of lactation. Furthermore, significant and positive correlations were found among the SL and DMY, contents of TS and TP. This means that contents of TS and TP, as well as DMY, increased with advancing lactation, which is in line with results reported by Sevi *et al.* (2000).

SUMMARY

Evaluation of the effect of parity, stage of lactation and interaction of PA x SL on daily milk yield, composition (contents of total solids, fat, total protein and lactose) and quality (somatic cell and bacteria counts) of organic milk of East Friesian sheep was carried out on organic farm in Habří in 2009. Ten ewes were on the second lactation and other ten ewes were on the third parity. All ewes were reared, till the end of our study, on permanent pasture and after weaning all ewes began to be machine-milked once a day. Samples for microbiological determination were milked by hand and were collected in sterile plastic containers. During the experiment, the daily feed ration of ewes consisted of permanent pasture (*ad libitum*), organic mineral lick (*ad libitum*) and organic oat (0.5kg/ewe/day). The milk records and samplings were carried out three times in the period from May to September, specifically on the average 75th, 132th and 190th day of lactation. The milk analysis were carried out using standard methods. The parity (PA) had no significant effect on all monitored indicators of milk, with the exception of somatic cell count (SCC). On the other hand, the stage of lactation (SL) had a significant effect on daily milk yield (DMY), contents of total solids (TS), total protein (TP) and lactose (L). Furthermore, the SL had a significant effect on total bacteria count (TBC), psychrotrophic bacteria count (PBC), total coliform count (TCC) and SCC. The evaluation of the interaction of the PA x SL was also an integral part of this study. The above mentioned factor had a significant effect on DMY, TS and TP contents and PBC. Under study, the milk protein was the most variable component of the milk. SCC as well as bacteria counts were very-well balanced on both lactations and relatively very low during the lactation period. Significant and positive correlations were found among the SL and contents of TS and TP. Furthermore, significant and positive correlation was observed between the PA and SCC, but on the other hand, significant however negative correlation was found between the SL and SCC.

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