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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

The effect of udder health on mineral concentrations and fatty acid composition of alpine goat milk*

Ágnes Sramek, Ákos Bodnár, Péter Póti, Ferenc Pajor**

Szent István University, Faculty of Agricultural and Environmental Sciences,
H-2100 Gödöllő, Páter Károly 1, Hungary

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The aim of the present study was to evaluate the concentrations of minerals and fatty acid content in milk of of multiparous Alpine goats (n=38). Milk samples for analyses were taken at four sampling times during the lactation period only in the evening milking (DIM: 56, 118, 196 and 224 days) from the fully milked udder, thus 152 milk samples were collected. Somatic cell counts (SCC) and milk constituents (fat, protein, lactose) were investigated from milk samples and the prevalence of pathogen bacteria presence was determined by bacteriological tests. In this study only mastitis free samples were analysed for their contents of minerals (Ca, K, Na, Mg, Zn and chlorine) and fatty acid composition. Samples were divided into two groups by somatic cell count: below 400.000 somatic cells/ml (n=19) and above 1.000.000 somatic cells/ml (n=20). The somatic cell counts have significant effects on milk composition, mineral and fatty acid contents of the milk. Milk quality parameters gradually changed in the samples above 1.000.000 cells/ml. Concentrations of the milk fat content, Na, Mg, and chlorine increased (P<0.05), while the lactose content and Zn concentration decreased. Furthermore, the udder health had great effect on the short and medium chain fatty acids *de novo* synthesis in the mammary gland. The unfavourable udder health status resulted in the reduced secretory activity of mammary epithelium, leading to lower concentrations of short and medium chain fatty acids in milk fat in the high SCC group. These results suggest that the higher SSC is associated with disadvantageous milk properties, which significantly reduced milk quality.

KEYWORDS: somatic cell count/ minerals/ fatty acids/ subclinical *mastitis*

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**Corresponding author: bodnar.akos@mkk.szie.hu

The demand for quality products made from goat milk is increasing worldwide, including Hungary. One of the reasons behind this trend is the nutritional value of goat milk, such as higher biological value of milk protein [Sanz Ceballos *et al*. 2009] and a more favourable fatty acid composition of milk fat than those of cow milk [Pajor *et al*. 2009]. In addition, the dairy goat sector is playing an important role in the agricultural economy in numerous parts of the world. Nevertheless, the quality of the product is essential in enhancing the competitiveness of goat milk production. Thus, not only nutritional value has to be qualified, but the hygienic quality (e.g. somatic cell count) of the milk as well. It is well known that a high somatic cell count (SCC) in dairy cattle is an indicator of udder health problems. In the milk of healthy cows the SCC does not reach 100.000 cells/ml [Juozaitiene *et al.* 2006]. In goats – according to Hinckley [1990] and Droke *et al.* [1993] the correlation is not so unequivocal. Majority of goats not showing symptoms of mastitis had a somatic cell count below $1x10^6$ cells/ml [Kuchtik *et al.* 2015, Leitner *et al.* 2016]. The threshold limit for goat milk by the US Food and Drug Administration is also $1x10^6$ SCC/ml [Paape *et al.* 2007]. In contrast, in the EU no admissible SCC limit has been established for goat milk. In another study the authors found no symptoms referring to mastitis at SCC over $5x10^6$ cells/ml [Dulin *et al.* 1983]. For example Hinckley [1990] explain these results with the apocrine type secretion of the alveolar cells in the udder. This secretion resulted in the appearance of nucleated or non-nucleated cytoplasm particles. Nucleated particles are included in the total somatic cell count [Paape and Capuco 1997].

However, increased somatic cell count is disadvantageous to milk yield and milk composition. The high level of somatic cell count in goat milk is associated with changes in protein, lactose, pH and electrical conductivity of raw milk and a decreased milk yield [Leitner *et al.* 2016, Bobbo *et al.* 2017 – in cattle; Kuchtik *et al*. 2015, Makoviczky *et al.* 2014 – in sheep; Leitner *et al.* 2004, Pajor *et al.* 2012 – in goat].

Although a majority of studies report the effect of udder health on milk yield, milk composition and other properties, there is only limited knowledge concerning the effects of subclinical mastitis on mineral concentrations and fatty acid composition of goat milk.

The aim of this study was to evaluate the effect of two somatic cell count levels (low level: <400.000 and high level: 1.000.000< cells/ml) on mineral contents and fatty acid composition of mastitis pathogen-free goat milk samples.

Material and methods

Experimental design

The study was carried out in a goat farm in the Pest County, Hungary. From a herd of about 120 polled goats, 38 multiparous Alpine goats with no signs of clinical mastitis symptoms (swelling, heat, redness or pain) were randomly selected and balanced for parity (2-3 number of lactation), time of kidding, kid rearing (8 week) and udder halves (homologous udder conformation).

Animals were milked from the beginning of April, kept indoors and fed on an alfalfa hay diet. During lactation the diets were adjusted to the NRC [2007] recommendations of energy and protein requirements to dairy goats. The animals were fed *ad libitum* medium quality alfalfa hay (NEl: 4.74 MJ/kg dry matter (DM.); crude protein: 183 g/kg DM) and additional concentrate (400 g/day) (NEl: 7.1 MJ/kg DM; crude protein: 180 g/kg DM) containing vitamins (A, D_3, E). A commercial trace-mineralized salt block was provided to goats. Goats were milked twice a day by machine milking in a milking parlour of the Westphalia type with 2×12 stands (vacuum: 48 kPa, pulsation ratio: 60:40, pulsation rate: 90/min).

Sampling procedure

Milk samples were collected at four sampling times only in the evening milking: in the first (days in milking, DIM: 56 days), in the second (DIM: 118 days), in the third (DIM: 196 days) and in the fourth (DIM: 224 days) part of their lactation, from the mixture milk of the fully milked udder for analyses, thus 152 milk samples were obtained.

All milk samples were tested in terms of somatic cell counts (SCC), milk composition (fat, protein, lactose) and the prevalence of udder pathogen bacteria presence based on bacteriological tests. Only mastitis pathogen-free samples (n=50) were used in this study. Milk samples with below 400.000 somatic cells/ml (n=19) and above 1.000.000 somatic cells/ml (n=20) were analysed for further investigations.

Milk analysis

From each milk sample, 0.1 ml milk was plated on Columbia esculin blood agar (Biolab Inc Budapest, Hungary) containing 5% sheep blood and 0.5 % esculin, and incubated at 37°C for 48 h. The isolates were identified as pathogen udder species by conventional methods, including Gram staining, colony morphology and haemolysis patterns.

The milk composition values (milk protein, milk fat, lactose) were determined using a LactoScope™ (Delta Instruments Ltd., Netherlands) device.

The Ca, K, Na, Mg and Zn levels were determined by Horiba Jobin Yvon Activa-M inductively coupled plasma – optical emission spectrometry (ICP-OES). Preparations of the milk samples (2.5 ml) were made in a Milestone Microwave Acid Digestion apparatus using 10 cm^3 65 % (v/v) nitric acid and 1 cm^3 35 % (v/v) hydrogen-peroxide. Wave lengths for each element were as follows: Na: 589.592 nm, K: 766.490 nm, Ca: 315.887 nm, Mg: 285.213 nm and Zn 213.857 nm.

Determinations for chlorine concentration of milk samples were described in the Hungarian Standard (1982).

The milk somatic cell count was determined using a Bentley FCM device at Livestock Performance Testing Ltd. (Gödöllő, Hungary).

Milk fat was dissolved in a sodium hydroxide-methanol solution and re-esterified to methyl-esters according to the AOAC [1990] method using boron trifluoride

(BF3). Methyl esters of fatty acids were determined by gas chromatography (gas chromatographer GC 2010, Shimadzu Kyoto, Japan) with a flame ionization detector (FID) and a column (CP-SIL-88, 100 m x 0.25 mm x 0.2 μm). The split injection ratio was 50:1. Helium was used as the carrier gas, applying a flow rate of 28 cm s-1. The split injection ratio was 50:1. The injector and detector temperatures were 270 and 300 °C, respectively. The oven temperature was held at 80°C for 0 min, then programmed at a rate of 2.5°C min-1 up to 205°C and held for 20 min and then increased again to 225°C at 10°C min-1, and held for 5 min (total time of oven program: 77 min.). Peaks were identified on the basis of the retention times for standard methyl esters of individual fatty acids (Mixture Me 100, Larodan Fine Chemicals AB, Limhamn, Sweden). The individual fatty acids were expressed as the ratio of their peak area of the sum of total fatty acids.

Statistical analysis

The effect of somatic cell counts was analysed as an independent variable. In the case of normal distribution (after verification by Shapiro-Wilk's test) the significance of differences was assessed by the t-test (. Otherwise variables were subjected to the Mann-Whitney U test. Statistical analysis was processed by the SPSS 23.0 software package [IBM Corp. 2015].

Results and discussion

The milk production, composition and somatic cell counts of the goats are shown in Table 1. The average fat and protein contents were 4.15% and 3.22%, while average milk yield was 685.34 kg. The average somatic cell content was 1.011.340 cells/ml with a minimum of 46.000 and a maximum of 2.968.000 cells/ml. The Alpine goats' milk production was similar to the Hungarian Sheep and Goat Breeder Association official data for Alpine goats milk performance in 2016 (average milk yield: 658.8 kg; length of lactation: 244.3 day) [HSGBA 2017].

Table 1. Chemical parameters of goat milk samples (n=152) and milk production of trial goats (n=38)

Items	Mean	SD	Minimum	Maximum
Lactation milk yield (kg)	685.34	93.51	480	825
Lactation length (day)	241.53	6.81	239	255
Daily milk yield (kg)	2.84	0.40	2.00	3.48
Milk fat (%)	4.15	2.18	1.68	12.43
Milk protein (%)	3.22	1.02	1.88	6.42
Lactose (%)	4.48	0.23	3.91	4.99
Somatic cell count (thousand/ml)	1011.34	244.08	42	2968

Table 2. Investigated parameters of goat milk samples by somatic cell categories

Items	Low level	High level	SEM	P
Lactose (%)	4.34	4.18	0.05	0.027
Milk fat (%)	3.04	3.82	0.23	0.009
Milk protein (%)	2.86	3.14	0.14	0.137
Na (mg/L)	300.50	373.79	23.66	0.017
K (mg/L)	949.00	811.14	70.19	0.143
Ca (mg/L)	1066.70	900.19	51.10	0.169
Mg (mg/L)	126.44	148.14	5.43	0.001
Zn (mg/L)	3.70	3.17	0.26	0.031
Chlorine (g/L)	1.77	1.94	0.03	0.022

The chemical and physical properties by somatic cell categories are shown in Table 2.

Values of fat, protein and lactose content are within the normal ranges for dairy goats reported by Park *et al.* [2006] and Kuchtik *et al.* [2015]. Milk fat and lactose content are altered significantly with increasing somatic cell content. Milk fat content considerably increased, while lactose content significantly decreased in the high level SCC group, corresponding with literature data [Sung *et al*. 1999, Leitner *et al.* 2004].

Table 2 shows the results of some mineral concentrations in goat milk. The mean values of Ca, K, Mg, Na, Zn and chlorine in milk were within the ranges reported by several authors [Park and Chukwu 1988, Park *et al.* 2006, Slacanac *et al.* 2011]. The influence of SCC level on milk Na, Mg and Cl concentrations was statistically significant. In our study the milk Na, Mg and Cl concentrations were higher in the high SCC group. These results were in concordance with the results of earlier reports by Holt [1985] and Ogola *et al*. [2007]. Those authors reported that the concentrations of Na and Cl were higher, while K and Ca were lower in infected cows' udder quarters. In the healthy udder the concentrations of Na and Cl are lower; K concentration is higher inside the cell and in milk compared to the extracellular fluid. Moreover, the Na and K concentrations are antagonistic inside the cell and the exterior fluid due to the action of an Na-K pump on the base membrane of the epithelium [Shennan and Peaker 2000]. In our study the results suggested that the increased somatic cell count has an effect on the membrane permeability with changes in the concentration of minerals in milk. Due to the higher SCC level, concentrations of Na, Mg and Cl were increased during secretion. Moreover, the milk Zn concentration is influenced by the udder health. Zn value was considerable lower in the high SCC category. Earlier some authors reported an advantageous effect of Zn, mainly organic Zn on milk quality and udder health, as Spain *et al.* [2005] showed that Zn proteinates might promote udder health and reduce intramammary infections throughout improved keratin synthesis in the teat canal.

Results of fatty acid analysis of milk samples by SCC categories are presented in Table 3.

Table 3. Fatty acid profile of goat milk by level of somatic cell count (% of total fatty acids)

Fatty acids	Low level	High level	SEM	P
C4:0	2.31	1.96	0.098	0.041
C6:0	2.56	2.17	0.084	0.005
C8:0	2.74	2.44	0.092	0.042
C10:0	9.96	8.61	0.310	0.007
C12:0	4.07	3.93	0.151	0.618
C14:0	10.29	10.13	0.200	0.629
C14:1	0.19	0.23	0.020	0.088
C16:0	33.07	30.61	0.753	0.048
C16:1	0.68	0.75	0.050	0.427
C18:0	6.68	6.62	0.287	0.896
C18:1n-9c	17.03	20.97	0.822	0.003
C18:2n-6	3.11	3.43	0.102	0.062
C18:3n-3	0.75	0.80	0.067	0.680
C20:2n-6	0.06	0.06	0.003	0.492
C20:4n-6	0.18	0.21	0.010	0.069
C22:0	0.14	0.12	0.018	0.689
C24:1	0.09	0.11	0.013	0.423
C16:0/C18:1	0.52	0.70	0.040	0.007
odd FA	2.10	2.50	0.093	0.009
Fatty acids according to origin				
16>	33.39	30.97	0.638	0.018
16	33.75	31.36	0.745	0.048
16<	30.02	34.57	0.981	0.006
de novo FA^{+}	50.27	46.65	0.754	0.004
SFA	77.06	72.51	0.891	0.002
MUFA	18.41	22.52	0.868	0.004
PUFA	4.54	4.97	0.145	0.071
desaturase index				
C14:1/(C14:1C14:0)	0.019	0.021	0.002	0.072
C16:1/C16:+C16:0)	0.020	0.022	0.002	0.157
C18:1/(C18:1+C18)	0.719	0.758	0.010	0.025

SFA – saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. $^{+}$C4-C14.

The udder health had a significant effect on the concentrations of short and medium-chain fatty acids (from C4 to C14) in milk, which were lower in the high SCC group. However, an important characteristic of goat milk fat is connected a high concentration of short and medium-chain fatty acids. The short-chain fatty acids, particularly caproic, caprylic and capric acids, affect flavour of dairy products [Zan *et al.* 2006]. Palmitic acid concentration changed with an increase in somatic cell counts. In the group with low SCCs palmitic acid concentration was higher compared to the other group. In contrast, the long-chain fatty acid concentrations were higher in the high SCC group. Short and medium chain fatty acids and approximately half of palmitic acid are synthetised in the mammary gland mainly from acetic acid, which

originates in the rumen. In our study all *de novo* synthesised fatty acids accounted for 50.0% in the low SCC group compared to the other group's 46.7%. Bauman and Davis [1974] reported that on the molar basis about a half of milk fatty acids are synthesised *de novo*. In contrast, the increased concentration of all 18-carbon and longer chain fatty acids in the higher SCC group may be due to the decreased substrate competition with short and medium chain fatty acids during milk fat synthesis [Storry *et al.* 1969]. The long-chain fatty acids (C16<) originate from blood throughout triglyceride mobilisation from the small intestine or from the adipose tissue. However, less than 10% fatty acids secreted in milk fat are mobilised from the adipose tissue [Bauman and Griinari, 2001]. In our study goats with a low somatic cell count had lower oleic acid (17.03%) and MUFA concentrations in milk (18.41%) in comparison to the goats from the high somatic cell count group (20.97% and 22.52%, $P<0.05$). Oleic acid is the main factor determining MUFA concentrations. This fatty acid is produced in mammary epithelial cells by the enzyme stearoyl-CoA desaturase (SCD). The SCD enzyme activity is generally estimated based on the Δ9 desaturase index. The Δ9 desaturase index is frequently expressed as the C14:1/(C14:1+C14:0), C16:/(C16:1+C16:0), C18:1c9/(18:1c9+C18:0) and CLA/(CLA+TVA) ratio. Among of these indices the C14:1/(C14:1+C14:0) ratio showed to be most closely related with the response of mammary Δ 9-desaturase activity (Bernard et al., 2008). In our study the SCC level has no significant effect on the C14:1/(C14:1+C14:0) and C16:/(C16:1+C16:0) ratios, but the C18:1c9/(18:1c9+C18:0) ratio was in higher in the high SCC group. Shingfield *et al.* [2010] reported that the SCD enzyme is responsible for 90% and only 60% of C14:1 and oleic acid (C18:1c9) synthesis in milk, with the rest originating from the diet throughout the digestive tract. In addition, the ratio of oleic acid to palmitic acid is important for milk producers, as a higher ratio of oleic acid to palmitic acid results in a softer fat [Dai *et al.* 2011]. Previously softer butter produced was reported from milk containing high concentrations of unsaturated fatty acids and reduced the starter culture activity in cheese [Ryhanen *et al.* 2005]. The ratio of oleic acid to palmitic acid was more unfavorable in the high SCC group; this value increased from 0.52 to 0.70 ($P<0.01$). Furthermore, odd-chain fatty acids were taken up by the mammary gland from plasma, similarly to long chain fatty acids. Odd-chain fatty acids mostly originated from the rumen, where they are produced by ruminal bacterial populations [Harfoot 1981]. The odd-chain fatty acids absorbed by the intestinal wall and uptake of their acids by the mammary tissue from blood lead to the presence of odd-chain FA in milk fat. The PUFA group, such as n-3PUFA, is important for consumers because of its beneficial effect on human health. In our study the PUFA concentrations were similar in both groups, although the level of n-6 PUFA was slightly higher in the high SCC group, while no difference in the milk n-3 PUFA content was observed. The lack of differences indicates that PUFA (mainly n-3 PUFA) secretion to milk by mammary epithelial cells was reduced. Our results suggest that the n-3 PUFA lipid fractions, such as phospholipids, were poorly taken up by the mammary gland. Nevertheless, an earlier preliminary report indicated that a high

n-3 PUFA intake has a beneficial effect on mammary cell integrity and inflammatory processes; α-linolenic acid supplementation helps to reduce the somatic cell count in mammary gland [Košmelj *et al*. 2001].

Summarizing, these results indicate that high SSC in raw goat milk is associated with disadvantageous milk properties, which may lead to a deterioration of milk quality. Subclinical mastitis damaged the udder tissue because of the destruction of the epithelial tissue. Our results suggested that a high level of SCC level had a considerable effect on milk composition and mineral contents. Concentrations of lactose, Zn and K decreased, while the concentrations of fat, Mg, Na and Cl increased in the high SSC group. This study showed that udder health has a great effect on the *de novo* synthesis of short and medium chain fatty acids in the mammary gland. For this reason the unfavourable udder health resulted in a reduced secretory activity of the mammary epithelium, leading to lower concentrations of short and medium chain fatty acids in milk fat in the high SCC group. Therefore, reducing somatic cell counts – apart from its veterinary aspects – is the basis for producing goat milk fulfilling its unique alimentary potential and the demand for high quality production.

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