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Evaluation of the behaviour and udder health parameters of horned and polled alpine goats in a Hungarian herd*

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The aim of the present study was to evaluate the temperament, milk somatic cell count and the prevalence of pathogen udder bacteria in multiparous horned (n=28) and polled (n=38) Alpine goats. The investigation was carried out in a commercial goat farm in the Pest county, Hungary. The temperament of 66 does was assessed using a 5-point-scale temperament test (1=very nervous, 5=very calm) during milking. The milk samples were taken at three sampling times during lactation, -only in the evening from the fully milked udder. Somatic cell counts (SCC) and the presence of pathogen bacteria species in milk samples were analysed. In this study, polled Alpine goats were found to be calmer than horned ones. Horned animals were more temperamental than polled animals (the score of 3.80 vs. 4.21; P<0.05). The percentage of calm animals was higher in the polled group; 86.8% of polled goats received scores of 4 and 5, while among horned animals it was only 75% (P<0.05). The presence of udder pathogens was significantly different between the horned and polled groups. Advantageous results were recorded in the polled group; the presence of udder pathogens was detected in only 47.4% of hornless animals. In contrast, the percentage of animals, in which udder pathogens were detected, was higher in the horned goats (65.5%; P<0.05). These results suggest that the polled animals were calmer and they have a more advantageous udder health status, which is associated with better milk quality.

KEYWORDS: goat / milk / somatic cell count / temperament / udder pathogens

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Nowadays, the demand for goat milk and dairy products is dynamically increasing both in Europe and worldwide. The main reasons and the background of this tendency is related with the remarkable nutritional value of goat milk compared to cow milk. The main milk producing goat breeds typically have hornless (polled) and horned genotypes. Recently, in goat husbandry an increased interest has been observed in the presence of horns, especially regarding the aggressive behaviour of the animals.

Loretz *et al.* [2004] found that space requirements at the feed barrier are higher in horned goats than hornless goats, whereas space requirements in the lying area were not influenced by the presence of horns in a loose housing system. In an earlier study by Margetinova *et al*. [2001] it was reported that horned goats were more aggressive than polled goats in milking parlour. It was also stated that horned goats' aggressive butting frequency was significantly higher than in hornless goats [Tölü and Savas 2007]. Moreover, the aggressive behaviour may influence the function of the immune system; Olfe *et al.* [2010] reported that nervous animals had lower serum concentrations of IgG and lymphocyte proliferation than calm animals. These results suggest that the impaired immune system function observed in more temperamental animals may be associated with the higher presence of udder pathogens in milk. Additionally, horned goats caused lesions and possible social stress to hornless goats [Waiblinger *et al.* 2011], and in horned dairy goats a higher risk of udder injuries was reported when compared to polled animals.

Handling of animals is important for farm management, since excitable, nervous animals are more susceptible to stress caused by routine handlings than calm animals [Voisinet *et al*. 1997]. The behavioural response to human handling [Burrow 1997] or to a novel environment [Sutherland *et al*. 2012] is described as temperament of animals. An unfavourable temperament is associated with lower milk production [Murray *et al*. 2009, Tóth *et al*. 2017] and immune system deficiency [Olfe *et al.* 2010]. Recently, many studies have been published on the behavioural aspects and reproduction in horned goats; however, still little is known about the temperament of dairy goats and to the best of our knowledge this is the first study to evaluate the effect of the presence of horns on the temperament score and udder health in goats.

The aim of this study was to investigate the effect of the presence of horns on the temperament, the incidence of udder pathogen bacteria species in milk and somatic cell count in Alpine goats.

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 and 60 horned goats, a total of 38 polled and 28 horned multiparous Alpine goats were randomly taken, without clinical mastitis symptoms (swelling, heat, redness or pain), and they were balanced for parity (2^{nd} (n=37) and 3^{rd} (n=29) parities), time of kidding, kid rearing (8 week) and udder halves (homogeneoushomologous

udder conformation). The polled and the horned animals were kept separately in deep litter pens. The minimal lying area was 2.0 m^2 per animal and the feeding place width was 40 cm per animal in each group.

Animals were milked from the beginning of April, kept indoors and fed an alfalfa hay diet. During lactation, the diets were adjusted to the NRC [2007] recommendations of energy and protein requirements for 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), which were offered during milking twice a day. A commercial trace-mineralised salt block and drinking water were provided *ad libitum* to the animals. Goats were milked twice a day with a machine milking in a Westphalia type milking parlour, with 2×12 stands (vacuum: 48 kPa, pulsation ratio: 60:40, pulsation rate: 90/min). Animals from the two groups were milked separately.

The temperament of goats was evaluated and milk samples were collected at the 56^{th}, 118^{th} and 196^{th} days of their lactation during the evening milking. The behaviour of animals was evaluated by an observer using a 5-point score system during the entire udder preparation and milking procedure [Budzynska *et al*. 2005] by direct scoring:

1) very nervous, continual and vigorous stepping and kicking;
2) continual and vigorous stepping, but no kicking;
3) occasionally vigorous leg movements;
4) calmly standing with few slight leg movements;
5) very calm, no leg movements.

Sampling procedure and analysis

Three milk samples (2 x 50 ml and 10 ml tubes) were taken for analyses from each goat at three sampling times immediately after temperament scoring, at evening milking: in the first period (days in milking, DIM: 56 days), in the second period (DIM: 118 days), and finally in the third period (DIM: 196 days) of the lactation, from the mixture milk of the fully milked udder.

Milk composition was determined from 50 ml samples (fat, protein and lactose) using the LactoScope™ apparatus (Delta Instruments Ltd., Netherlands), while milk somatic cell count was determined using a Bentley FCM device at Livestock Performance Testing Ltd. (Gödöllő, Hungary).

From a 10ml tube, 0.1 ml milk was plated on Columbia esculin blood agar (Biolab Inc., Budapest, Hungary) containing 5% of 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, according to the National Mastitis Council guidelines (NMC, 1999).

Polled and horned animals were divided into four groups based on the occurrence of the udder pathogen bacteria at each sampling time:

1 – negative samples;
2 – minor pathogen bacteria species appeared once;
3 – minor pathogen bacteria species appeared twice;

4 – minor pathogen bacteria species appeared at each of the three sampling periods.

Statistical analysis

Statistical analysis was conducted using the SPSS 25.0 software package (IBM Corporation, Armonk, NY, USA). The Kruskal-Wallis test was used to assess differences among temperament scores in the lactation stages. The impact of parity number and the presence of horns on temperament scores was evaluated by the Mann-Whitney U test. The prevalence of udder pathogens between polled and horned goats was examined using PearsonChi^2 test.

Prior to the analysis, Shapiro-Wilk's test was used to verify the normality distribution of the somatic cell count (SCC) data. Subsequently the log transformation of SCC values was applied. . Statistical analysis was carried out in order to determine the effect of lactation stage, parity number and the presence of horns (fixed effects) on somatic cell counts. The general linear model (GLM) procedure was used to analyse variance. The statistical model was as follows:

$$y_{ij} = \mu + LS_i + PN_j + PH_k + e_{ijk}$$

where:

y_{ij} – the value of the dependent variable;

μ – the overall mean;

LS_i – the effect of lactation stage;

PN_j – the effect of parity number;

PH_k – the effect of the presence of horns;

e_{ijk} – the random error.

The Tukey HSD post-hoc test was used for pairwise comparisons. The parity number × litter size interactions were not significant for the somatic cell count and only the main effects are presented in the Results.

Group means of logarithmic somatic cell counts depending on the presence of horns were compared using the F-test and Student's t-test. ANOVA analysis was applied to study differences in somatic cell counts of milk in terms of the occurrence of udder pathogens.

Results and discussion

The lactation stage had a significant effect on somatic cell counts of milk; the lowest somatic cell count was found in the early lactation stage compared to the other stages of lactation, which is in line with the results of earlier studies [Wilson *et al.* 1995] (Tab. 1). In contrast, temperament scores were stable at different stages of lactation in both groups. Parity had no effect on the temperament score and the somatic cell count, similarly as it was with milk yield, fat and protein contents of milk (not presented in the Table) (2nd parity: 610kg, 3.46% and 2.99%; 3rd parity: 625kg, 3.42%

and 3.01%). Parity has a marked effect on milk production in goats; goats in the first or fourth and successive parities had lower milk yields than goats in the second or third parity. Previously several authors reported no differences between the second and third parity for milk yield, milk composition and somatic cell count [Rota *at al.* 1993, Arnal *et al.* 2018, Zamuner *et al.* 2019]. Moreover, there was no significant interaction between lactation stage and parity in the case of temperament scores and somatic cell count. The presence of horns had a significant effect on temperament scores of goats ($P<0.05$). In contrast, no significant difference was observed between the two groups regarding their somatic cell counts.

Results of temperament scores for polled and horned Alpine goats are presented in Table 2. The polled Alpine goats were significantly calmer (score of 4.19) than the horned ones (3.80). In our study, 42.2% of polled Alpine goats received the score of 5 points (calm animals), 44.7% of goats got the score of 4, 7.9% of goats the score of 3 and 2.6% of goats the score of 2 points, respectively. In turn, 14.3% horned Alpine goats received the score of 5 (calm animals), 60.7% of goats the score of 4, 21.4% the score of 3 and 3.6% the score of 2. No animals with the score of1 were found in either of the groups. The repeatability of temperament scores was about 0.31-0.44 [Halloway and Johnston 2003]. This suggested that multiple measurements are necessary; in this study, evaluation repeated three times may be sufficient for adequate results. The ratio of calm animals in the polled and horned groups was large; 86.8% of polled goats received 4 or 5 points, while in the horned animals it was only 75% ($P<0.05$). The mean percentage of calm animals (scores of 4+5 points) was 81.8%. Therefore, the proportion of goats with an unfavourable temperament score during milking was relatively low (less than 20%). Similar results had been reported earlier by Póti *et al*. [2015], who found that approx. 80% of dairy Alpine goats were calm, and by Tóth et al. [2017], who reported that 85% of dairy sheep were calm.

Table 1. Main effects of lactation stage (LS), parity number (PN) and presence of horns (PH) on temperament scores and milk somatic cell count of Alpine goats (n=66)

Item	LS			PN		PH		Total	SEM	P		
	1	2	3	1	2	polled	horned			LS	PN	PH
Temperament score	3.94	4.14	4.04	4.08	3.95	4.19	3.80	4.03	0.072	0.509	0.347	0.006
Somatic cell count (log/ml)	5.69^{a}	6.05^{b}	5.98^{b}	5.93	5.88	5.94	5.86	5.90	0.027	<0.001	0.206	0.111

Earlier studies indicated that horned goats are more aggressive compared to polled goats and thus need more effective feed barriers than polled animals [Margetinova *et al.* 2001; Loretz *et al.* 2004]. In our study, the size of feed barriers and the lying area met the recommendations [Toussaint 1997]. In addition, Waiblinger *et al.* [2011] reported that horned dairy goats have a higher risk of udder injuries than polled animals.

Table 2. Numbers of polled and horned goats according to mean temperament score categories

Temperament score categories	Polled (n=38)		Horned (n=28)		P+
	n	%	n	%	
2	1	2.63	1	3.57	0.700
3	4	10.53	6	21.43	0.083
4	17	44.74	17	60.71	0.034
5	16	42.11	4	14.29	<0.001
4+5	33	86.84	21	75.00	0.047

Table 3. Prevalence of udder pathogens in milk samples by presence of horn

Mastitis pathogens	Polled	Horned	Total	P+
Negative	52.6	34.5	44.95	0.015
Infected samples	47.4	65.5	55.05	0.010
Pathogens from infected samples				
CNS	68.5	78.2	73.4	0.200
Corynebacterium sp.	22.2	12.7	17.4	0.137
CNS + *Corynebacterium* sp.	9.3	9.1	9.2	0.910

CNS – Coagulase-negative *Staphylococcus.*

The prevalence of udder pathogens in milk samples depending on the presence of horns is presented in Table 3. The mean prevalence of udder pathogens was 55%, which corresponds with earlier results published by Kalogridou-Vassiliadou [1991], Delano *et al*. [2002] and Pajor *et al.* [2016], whereas it was higher compared to other studies [Bagnicka *et al*. 2011]. However, the prevalence of udder pathogens was markedly different between the horned and polled groups. The most favourable value was found in the polled group. Prevalence of udder pathogens in the polled group was only 47.4%, while the analogous value in horned animals was 65.5% ($P<0.05$).

Two types of minor udder pathogens were identified – Coagulase-negative *Staphylococcus* (CNS) and *Corynebacterium* sp. However, the combined occurrence of these udder pathogens was detected in a small proportion of the analysed samples. In our study, CNS was the most prevalent minor mastitis pathogen; this corresponds with previous reports [Bagnicka *et al.* 2011]. The mean prevalence of CNS bacteria was 73.5%; the difference between the two groups was not marked, as the CNS was 68.5 % in the polled and 78.2% in the horned group. Earlier such authors as Bagnicka *et al*. [2011] and Souza *et al.* [2012] reported that the presence of CNS mastitis pathogens caused an increase in the total somatic cell counts in dairy goats.

The mean prevalence of *Corynebacterium* sp. from infected samples was 17.4%. It was similar to the results of Kalogridou-Vassiliadou [1991] and Bagnicka *et al.* [2011]. A slight difference was detected in the investigated groups; *Corynebacterium* sp. was found in 22.2% samples in the polled group, while it was 12.7% in the other group. Nevertheless, no major mastitis pathogens were found in milk in our study . It is well known that these bacteria have a great impact on udder health; the presence

of these bacteria increase the somatic cell count in dairy animals, as presented in earlier reports [Contreras et al. 2003]. Lack of major mastitis pathogens shows that the protection against this type of pathogens is satisfactory on this farm.

Polled and horned animals were divided into four groups based on the occurrence of the udder pathogen bacteria during the sampling period (Tab. 4). In the polled group, 16% of total animals have all samples free form mastitis pathogens, while 37% of goats had mastitis pathogens detected only in one sample. Altogether 44% of animals were found to have two samples, while nearly 3% of polled goats had all the three samples positive for mastitis pathogens, with the samples collected individually during the sampling period. In contrast, there were no negative samples taken from any horned goats. A total of 25% horned animals had only one sample infected by udder pathogens and close to 54% of animals had seriously infected samples. Additionally, more than 21% of horned goats had all the three samples positive for mastitis pathogens during the sampling period.

Table 4. Occurrence of mastitis pathogens during three sampling times and relationship to percentage of goats

Number of positive samples	Polled (n=38)		Horned (n=28)		P^+
	n	%	n	%	
0	6	15.8	0	0	<0.001
1	14	36.8	7	25.0	0.093
2	17	44.7	15	53.6	0.258
3	1	2.6	6	21.4	<0.001

Table 5. Occurrence of mastitis pathogens during three sampling times and relationship to somatic cell count in milk of goats

Number of positive samples	Polled	Horned	SEM	P^+
0	5.78 (n=6)	- (n=0)	0.056	-
1	5.94 (n=14)	5.76 (n=7)	0.058	0.160
2	5.99 (n=17)	5.89 (n=15)	0.037	0.169
3	5.86 (n=1)	5.91 (n=6)	0.087	-
P	0.184	0.505		

The mean somatic cell count was 796 000 thousand cell/ml (5.90 log cell/ml) (see Tab. 1), 5.94 and 5.86 log cell/ml for the polled and horned animals. Milk somatic cell counts were consistent with previous studies [Sramek *et al.* 2018, Kuchtík *et al.* 2015]. The healthy goats' somatic cell count does not reach 1 million cells/ml, in this case most of the animals showed no symptoms of mastitis [Leitner *et al.* 2016].

The number of sampling times in which mastitis pathogens were identified was not associated with somatic cell count; moreover, no differences were found in somatic cell counts between the polled and horned goats (Tab. 5). However, only in the polled group uninfected goats had significantly lower somatic cell counts (5.78 log cell/ml) than those, which had any infected samples (5.97 log cell/ml; $P<0.05$)(not presented

in Table). Therefore, any occurrence of udder pathogens during the sampling period increased significantly the somatic cell count in goat milk. This corresponds with the results of Contreras *et al*. [2003], Luengo *et al*. [2004] and Pajor *et al.* [2016].

In conclusion, polled Alpine goats were calmer than horned ones. The proportion of calm animals (scores of 4+5) among polled goats was higher (87%) compared to horned animals (75%; $P<0.05$). Polled goats had a lower prevalence of identified udder pathogen bacteria in comparison to horned animals. These results suggest that horned goats are more susceptible to udder health problems.

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