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ANALYSIS OF THE CAEV INFECTION IMPACT ON THE MILK YIELD AND MILK SCC OF POLISH DAIRY GOATS

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ABSTRACT

Goats' milk is an attractive product for producers and consumers, because of its health benefits and chemical composition. But there arestill no strict regulations according to specific hygienic rules for this milk safety. Somatic cells number, which is the basic parameter for bovine milk (SCC) vary in goats and it is affected by many factors, e.g. age, estrous phase, type of milking, but also by infectious factors. In our experiment we had analyzed the potential influence of CAEV infection on dairy goats productivity and milk SCC. The experiment was conducted on 24 individuals (12 seropositive/12 seronegative) , with milk samples analysis during subsequent lactations (from 2nd to 5th). The results have shown the impact of viral infection on early and late lactations, with decreased milk yield & increased number of somatic cells in milk. We conclude that it could be correlated with infection progression and the efficiency of goats' immune system.

Keywords: Goat, milk, milk somatic cells, CAEV

INTRODUCTION

In Poland a goat was once a symbol of poverty and help in crisis, nowadays products from goat's milk are high-end goods. Goat'smilk, currently is associated with a luxury and therefore its products can be sold at significantly higher prices than similar products from cow's milk. Also the increase of consumer's awareness and in hence demand for products from goat's milk encouraged producers to increase production, and offering handicraft food products made from the milk of animals kept on organic farms. This serves to meet the needs and tastes of consumers. Goat's milk is an attractive product, not only for consumers but also for producers. For consumers, due to the high degree of digestibility, the preferred chemical composition, which in this respect is more similar to human milk than cow's milk. A growing rate of consumers has a knowledge about the low allergenicity of goat's milk, which can be an alternative for people allergic to cow's milk (**Lara-Villoslada *et al*., 2004**). It is estimated that in the population of children and infants 5% to 15% of children have symptoms suggesting hypersensitivity to cow's milk protein and 2 - 7.5% of the children has an allergy to cow's milk. There is a very broad spectrum of symptoms observed, and sometimes strong intolerance and allergies to cow milk. Started with the symptoms associated with the digestive tract (abdominal pain, bloating, or more or less expressed diarrhea), through the skin lesions (pruritus and rash), symptoms of respiratory tract (rhinitis) or even anaphylaxis. The amount of cow's milk, which can cause immediate reactions vary from one drop to 161 ml. (**Vandenplas *et al*., 2007, Dias *et al,* 2010**). Several studies have reported real benefits from the use of goat milk as an alternative for cow's milk allergy. (**Lara-Villoslada *et al*., 2004**)

In Poland, according to the Statistical Yearbook 2012, the goat population in 2011 was about 111,8 thousand units (http:// www. stat. gov. pl/ cps/ rde/ xbcr/ gus/ rs_ rocznik_ rolnictwa_ 2012. pdf), in 27785 farms. In Poland,most of thegoatsarekepton agrotouristic farms. In 2010,81.5% of goats herds holdingshad1-4animals, representing 41.6% of the total population ofgoats.(Agricultural Census 2010 Livestock andselected elements ofthe methodsof animal productionhttp://www.stat.gov.pl/gus/5840_12396_PLK_HTML.htm).In Poland, themaindirection of goats useis milk production.

The level of milk production and changes in the goat mammary gland during the lactation cycle, depends primarily on the number of epithelial cells involved in the milk synthesis, a balance between the rate of epithelial cells proliferation, their apoptosis and the secretory activity of these cells (**Safayi *et al.*, 2010**). It was noted that the next lactations in goats are characterized by a higher milk yield (20%), although are shorter in duration, compared with animals that are in the first lactation. This is related to e.g. a lower percentage of mammary epithelial cells (MEC -mammary epithelial cells) undergoing apoptosis process, compared with the multiparous mammary gland. This means that in the MEC population in primiparous goat's mammary glands, cells, which proliferated during lactation were more persistent, and thus the time of their secretory activity significantly increases, which automatically means a longer period of lactation. **Safayi***et al* in their studies suggest that it is related to higher activity of antiapoptotic protein bcl-2 than activity of proapoptotic factor bax (BCL2-associated X protein), in the regulation of apoptosis after weaning and kid feeding. They also proved that the beginning of lactation in multiparous goats is associated with activation of PRLR (prolactin receptor) in the MEC and the start of abundant milk secretion, including whey protein LALBA (α-lactalbumin).The expression of these factors was highest during parturition in multiparous goats.

Somatic cell count (SCC) in goat milk is affected by many non-infectious factors, including animals age, estrous phase, the herd size, stress, type of milking, udder and teat structure, lactation stage and parity, and the type and availability of food (**Ollier *et al.*, 2007**, **Safayi *et al.*, 2010**). **Zengend Escobar (1996)** also observed differences in the number of somatic cells obtained from different breeds of animals, during a full duration of lactation. The goat milk somatic cells consist of three groups: epithelial cells, leukocytes, and fragments of cytoplasm. SCC in goat's milk varies during lactation cycle. Polymorphonuclear cells (PMN) comprise the main cell type found in the goat's milk - 45-74%. 15-41% a pool of somatic cells there are macrophages, and lymphocytes cover 9-20% of cell population in the milk derived from healthy mammary gland (**Paape *et al.*, 2007**). Mononuclear cells from goat mammary epithelium are producing chemotactic factors for PMN, neutrophils, macrophages and lymphocytes, which contributes to increasing their migration to the milk, the process is particularly exacerbated in late lactation, as increased natural protection of udder during involution. Chemotactic factors are therefore a physiological regulator of the mammary gland homeostasis (**Manlongat *et al*., 1998**).

In the United States authorized number of somatic cells in goat milk was determined by the FDA at a level 1 x10^6/ml(http:// www. fda. gov/Food/ Food Safety/ Product-Specific Information/ Milk Safety/ default. htm). In Poland, same as in the whole European Union, under Directive 92/46 ECC (1992) in Regulation (EC) No 853/2004 of the European Parliament and of the Council

laying down specific hygiene rules for the hygiene of foodstuffs, to permit the use for the manufacture of certain dairy products raw milk not meeting the criteria laid down in Annex III, Section IX, as regards its plate count and somatic cell count. In Section IX, Chapter I, Part III, paragraphs 1-3 are given statutory requirements in relation to raw cows' milk: somatic cell count ≤ 400 000/ml , while for raw goat milk, there is no absolute value for somatic cell count. Food business operators must initiate procedures to ensure that raw milk meets the following criteria for raw milk from other species than cow, plate count at 30 °C (per ml) ≤ 1500000 as rolling geometric average over a two-month period, with at least two samples per month.

Although the severity of somatic cells in goat milk in autumn is usually a physiological phenomenon, it must be remembered that infection with CAE (caprine arthritis encephalitis) also has an impact on the increase of SCC in the goat's milk. (**Paape, 2007**) CAE is a chronic disease occurring mostly in goats, with sheep being sporadically affected. CAE was described at the first time in the United States, in 1974 (**Cork *et al.*,1974**). Till now CAE has been reported from all over the world (Adams et al., 1984), in Poland was confirmed in 1996 (**Kaba *et al.*, 2010**). It is caused by caprine arthritis-encephalitis virus (CAEV) - single-stranded RNA virus belonging to the family Retroviridae, genus Lentivirus. Closely related lentivirus is responsible for maedi-visna disease (MV) in sheep. For many years following their isolation in 1960 for MVV and 1980 for CAEV they had been recognized as distinct pathogens, infecting two different ruminant species – sheep and goats, respectively (**Sigurdsson *et al.*, 1960**, **Crawford *et al.*, 1980**). Virus infects monocytes where, thanks to the enzyme – reverse transcriptase – it changes into DNA provirus and becomes integrated into the host genome (**Zink *et al.*, 1990**). Such latent infection is life-long and persists despite vigorous humoral immune response mounted by the host usually in 2 to 8 weeks after infection. Intensively produced neutralizing antibodies are incapable to eliminate the virus, although they markedly reduce its load. The virus is able to evade the humoral immune response by making various antigenic variants of itself (**Perk**, **1995**). Infected monocytes migrate to the various tissues, mainly synovium, lungs, udder and central nervous system, where they differentiate into macrophages. Virus replication takes place exclusively at this moment. Infected macrophages secrete inflammatory cytokines which attract lymphocytes and induce chronic immune-mediated inflammation in infected tissues (**Zink *et al.*, 1990**; **Haase**, **1986**; **Phelps *et al.*, 1993**). As it is a very slow process, the disease develops slowly with clinical manifestation not sooner than 12 months after infection (**Smith *et al.*, 2009**). The disease may be easily transmitted from infected does to suckling kids via colostrum. Nevertheless, horizontal transmission occurs as well, although it requires long direct and indirect contact between goats (**Rowe and East, 1997**; **Blacklaws *et al.*, 2004**).

The aim of this study was to determine the effect of CAE infection on the daily performance of dairy goats and the somatic cells number in the milk of goats free of viral infection and infected with CAE in the one herd.

MATERIAL AND METHODS

The study was conducted on 24 Polish White and Fawn Improved (PWI and PFI) dairy goats, selected from 50 goats, maintained in the herd belonging to the Experimental Farm in the Institute of Genetics and Animal Breeding in Jastrzębiec, Poland. The animals were fed according to the INRA system (Jarrige, 2002). Water was available ad libitum. Goats were machine-milked twice a day.

Two analogous groups of goats according to the breed and age: control (healthy goats) and experimental (infected goats) were distinguish (N=12 in each of them). Goats were between the second and fifthlactation. Choosing of animals to particular group was based on results of at least two serological ELISA tests conducted not less than 12 months apart and microbiological status of mammary gland. The serum samples were tested for antibodies against SRLV with ELISA test (IDEXX CAEV/MVV Total Ab Screening Test). The tests were performed according to the manufacturer's protocols using ELISA reading device ICN Flow TitertekMultiscanPlus Mk11 (Labsystems, Espoo, Finland)

Sampling of milk probes. Milk samples (300 ml) were collected from animals free of clinical mastitis, during the morning milking, five times at regular intervals during lactation (or: every 60 days throughout the lactation period, during the morning milking) (1th trial in 7 - 10 days after parturition). Samples were immediately refrigerated and transported on ice (4° C) to the laboratory. As soon as possible the milk samples have undergone the procedure of somatic cells obtaining. All of milk samples were screened by microbiological testing and only pathogen-free animals were used in the trial. Isolated pathogens were identified using biochemical tests API. (**Malicki** and **Binek2004**). Only pathogen-free animals were used in our studies.

The total somatic cell count (SCC) was estimated by an automated fluorescent microscopic somatic-cell counter BactocountIBCm (Bentley Instruments, USA), which counts only cells containing DNA stained by ethidium bromide.

Statistical analysis.The variance analysis was conducted using the GLM procedure with the Kramer-Tukey adjustment of SAS package (SAS/STAT 2002–2003). Before statistical analysis, the total SCC were transformed to natural logarithm values and expressed as somatic cell score (SCS).

RESULTS AND DISCUSSION

The presented study included animals from 2nd to 5th lactation. Primiparous goats were not included in the study due to lack of CAE seropositive young individuals. In our study we did not observed a significant decrease in milk production and a substantial increase in the level of somatic cells in the milk of goats infected with CAE in each subsequent lactations.

A slight increase in the number of somatic cells in the milk of seronegative goats in comparison with seropositive goats in 3rd and 4th lactation was statistically not significant (Fig. 2).

Statistically significant decreasing were found in milk yield in 2nd (at the beginning of infection) and 5th lactation (after long lasting infection), and simultaneously the increase in the number of milk somatic cells in 2nd and 5th lactations. (Fig. 2). Despite the high differences in average SCC during 3rd and 4th lactations between investigated groups, high variation in SCC probably caused the lack of statistically proved differences.

The increase in the number of somatic cells in the milk of both healthy and sero-positive goats in the 240th day of lactation in all studied lactation groups was associated with a physiological increase in SCC (Tab. 2), as a result of the estrous cycle phase and mammary gland preparing to drying off phase. **Leitner *et al.*, (2010)** reported similar milk yields in CAEV seropositive and seronegative does in their second or further lactations and found that milk yield was greater in seronegative animals in first lactation. And **Martinez *et al.*, (2013)** actually showed a decrease in milk production in seropositive goats from the 2nd to the last lactation.

Our study showed overall decrease in milk yield of goats infected with CAEV in comparison with similar group of seronegative goats (Tab. 1). On the contrary, **Kaba *et al*,.** (2012) in their long-term observations, did not show differences between the seropositive and seronegative animals from the trail. By the analysis of these discrepancies should be taken into account that our goats group was selected and had 24 individuals (12 healthy and 12 with CAE in the same lactations) and we had analyzed only the results of the morning milking in selected five points during lactation in one year. Moreover, for the analysis were taken only the results of milk samples free of pathogenic bacteria. **Kaba *et al* ,** (2012) have observed the milk yield in dairy goats for 12 years in the 10 months of the year, and the analysis was performed on milk samples both, from morning and afternoon milking, as the daily test performance, without examination of the pathogen bacteria presence. Moreover, the analyses were conducted for all parities together, with taking into account in a calculation model the lactation number as fixed effect. In our studies small number of individuals may have an impact on large variation within milk yield.

Several previous studies showed a strong influence of CAEV infection on SCC (**Ryan *et al.*,1993**; **Nord** and **Adnoy**, **1997**; **Turin *et al.*, 2005**). However, **Nord** and **Adnoy** (**1997**) observed the increase of SCC only in 2-yr-old does, not in primiparous does, whereas **Turin***et al.* (**2005**) noted elevation of SCC in primiparous females. The most recent studies(**Leitner***et al.*, **2010**, **Kaba***et al.*, **2012**) yielded results consistent with ours: no relationship between the infection and SCC was found. It has been postulated that SCC cannot be the only indicator of the udder infection in goats (**Bagnicka *et al.*, 2011**). Infections were found to account for less than 10% of the variation in milk SCC, whereas increasing DIM, month of the year, and parity were most important (**Wilson *et al.*, 1995**).

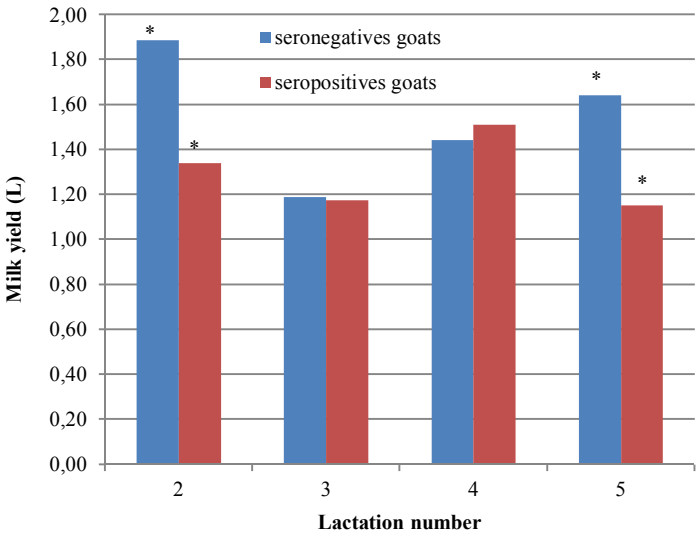

Figure 1 Average (Mean) daily milk yield (kg) according to caprine arthritis and encephalitis virus serostatus and subsequent lactations (Polish dairy goats)

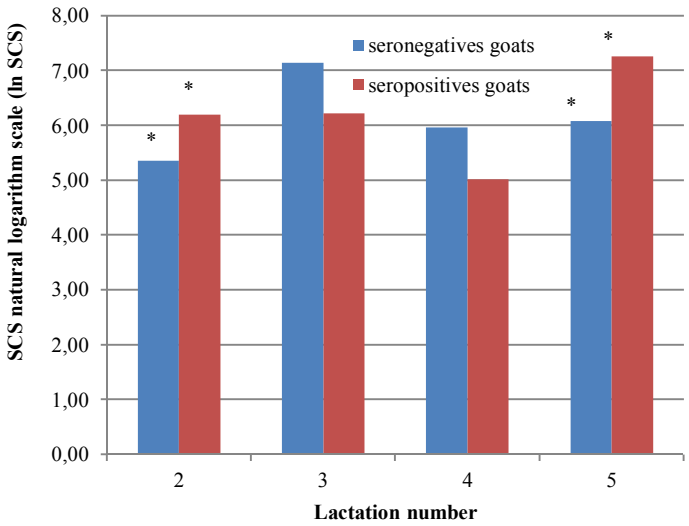

Figure 2 Mean SCS natural logarithm scale (ln SCS)according to caprine arthritis and encephalitis virus serostatusand subsequent lactations. SCS - somatic cell score

Table 1 Lactation-specific daily milk yield (L) according lactation number / day of lactation to animal caprine arthritis and encephalitis virus serostatus (Polish dairy goats)

Lactation number	CAEV	LactationDay	Milk yield (L)	(SE)
2	Seronegative	10	1.05000000 ^{A}	0.31736491
		70	1.90000000 ^{B}	0.31736491
		130	2.00000000 ^{B}	0.25912736
		180	2.13333333 ^{B}	0.25912736
		240	2.13333333 ^{B}	0.25912736
	Seropositive	10	0.6000000 ^{A}	0.44882176
		70	1.30000000	0.44882176
		130	1.30000000	0.44882176
		180	1.80000000 ^{B}	0.44882176
		240	1.70000000 ^{B}	0.44882176
3	Seronegative	10	0.80000000	0.44882176
		70	1.40000000	0.44882176
		130	1.30000000	0.44882176
		180	1.40000000	0.44882176
		240	1.20000000	0.44882176
	Seropositive	10	0.83333333	0.25912736
		70	1.20000000	0.25912736
		130	1.23333333	0.25912736
		180	1.43333333	0.25912736
		240	1.30000000	0.25912736
4	Seronegative	10	0.86666667 ^{a}	0.25912736
		70	1.50000000 ^{b}	0.25912736
		130	1.70000000 ^{b}	0.25912736
		180	1.86666667 ^{b}	0.25912736
		240	1.30000000 ^{b}	0.22441088
	Seropositive	10	1.00000000	0.31736491
		70	1.70000000	0.44882176
		130	1.50000000	0.44882176
		180	1.80000000	0.44882176
		240	1.60000000	0.44882176
5	Seronegative	10	1.03333333 ^{a}	0.25912736
		70	1.60000000	0.25912736
		130	1.77500000	0.22441088
		180	1.95000000	0.22441088
		240	1.86666667 ^{b}	0.25912736
	Seropositive	10	1.13333333	0.25912736
		70	1.50000000	0.31736491
		130	1.20000000	0.31736491
		180	1.20000000	0.31736491
		240	0.50000000	0.31736491

Legend: CAEV - caprine arthritis and encephalitis virus, ln SCS - SCS natural logarithm scale, ^{AB}- comparing seropositive and seronegative goats of same lactation group at $p \leq 0.01$; ^{ab} - comparing seropositive and seronegative goats of same lactation group at $P \leq 0.05$

Table 2 SCCin natural logarithm scaleaccording to lactation number and day of lactation to animal caprine arthritis and encephalitis virus serostatus (Polish dairy goats)

Lactation number	CAEV	LactationDay	ln SCS	standard error (SE)
2	Seronegative	10	3.69416393 ^{A}	0.78256918
		70	4.92213045	0.55335997
		130	4.94358041	0.63896506
		180	4.88012449	0.63896506
		240	7.14186054 ^{B}	0.63896506
	Seropositive	10	5.67332327	1.10671995
		70	6.15909539	1.10671995
		130	6.25766759	1.10671995
		180	5.38907173	1.10671995
		240	7.53689713	1.10671995
3	Seronegative	10	6.55393340	1.10671995
		70	6.84054653	1.10671995
		130	7.39079852	1.10671995
		180	7.08757371	1.10671995
		240	7.12044437	1.10671995
	Seropositive	10	5.77655719	0.63896506
		70	6.53379393	0.63896506
		130	5.80636560	0.63896506
		180	5.75362215	0.63896506
		240	6.88407418	0.63896506
	Seronegative	10	5.12030585 ^{a}	0.63896506

Lactation number	CAEV	LactationDay	ln SCS	standard error (SE)
4		70	5.24539694	0.63896506
		130	5.32225948	0.63896506
		180	5.00378373	0.63896506
		240	7.69537781^{b}	0.55335997
	Seropositive	10	3.43450723^{a}	0.78256918
		70	5.38449506	1.10671995
		130	4.45434730	1.10671995
		180	4.91998093	1.10671995
		240	7.37086017^{b}	1.10671995
5	Seronegative	10	4.61302361^{a}	0.63896506
		70	5.31618107	0.63896506
		130	6.04542536	0.55335997
		180	5.62018560	0.55335997
		240	7.65835324^{b}	0.63896506
	Seropositive	10	5.76952353^{A}	0.63896506
		70	7.09369565^{a}	0.78256918
		130	7.23624630^{a}	0.78256918
		180	7.08792987^{a}	0.78256918
		240	9.32525910^{Bb}	0.78256918

Legend: CAEV - caprine arthritis and encephalitis virus, ln SCS - SCS natural logarithm scale, ^{AB}- comparing seropositive and seronegative goats of same lactation group at $p \leq 0.01$; ^{ab} - comparing seropositive and seronegative goats of same lactation group at $P \leq 0.05$

CONCLUSION

On the base of our preliminary research we are trying to interpret the phenomenon of the impact of CAE infection on the functioning of the mammary gland of dairy goats. Based on the present results it appears that the goats react immediately after the infection and it is manifested by decreased milk yield and increased number of somatic cells in milk. This is probably connected with spreading and multiplication of the virus in the mammary gland, and destruction of galactopoietic cells. After some time, the organism's resistance increases and the negative effect of the virus is reduced. Only in older individuals (in our study, 5th lactation), whose immune system is getting weak, again we can see the impact of CAEV infection on goats productivity.

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