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N-acetylcysteine supplementation may affect somatic cell count in goat milk (short communication)

Abstract

The aim of this study was to estimate an influence of supplementation of exogenous N-acetylcysteine (NAC) on the somatic cell count (SCC, quantified as cells per ml) and chemical composition of goat milk. The study was carried out on 15 goats, in the last part of lactation (220-250 days). The animals were divided into 3 groups according to SCC in their milk: 1st – up to 2×10^6, 2nd – from 2 to 4×10^6 and 3rd – above 4×10^6. The animals were clinically healthy. They were given NAC in the amount of 12 mg/kg of live body weight *per os* for 7 days, once a day, during evening milking. Milk yield, as well as fat, protein and lactose contents and SCC were studied three times: 1st – just before starting the experiment, 2nd – after 7 days of NAC application and 3rd – a week after the end of NAC application. The analysis of variance with General Linear Model (GLM) procedure of SAS® package was used. There was observed the significant decrease of SCC after one-week supplementation of NAC and stagnation of SCC after next week in all three groups. The changes of chemical composition of milk were not significant.

The significant decrease of SCC may indicate improvement of health of mammary gland. The oxidation processes might be decreased and probably the increase of immune defence of organism took place. One can suggest that the supplementation of NAC may lead to improvement of goat udder health.

Keywords: N-acetylcysteine, somatic cell count, goat milk

Zusammenfassung

Titel der Arbeit: **Der Zusatz von N-acetylcysteine zum Futter könnte die Anzahl der somatischen Zellen in Ziegenmilch beeinflussen** (Kurzmitteilung)

Zweck der Untersuchungen war es, den Einfluss der N-acetylcysteine (NAC) auf die Anzahl der somatischen Zellen sowie auf die chemische Zusammensetzung der Milch zu erforschen. Die Untersuchungen wurden an 15 Ziegen im Endstadium der Laktation (220-250 Tage) durchgeführt. Die Ziegen wurden in drei Gruppen aufgeteilt, die sich durch die Anzahl der somatischen Zellen per Milliliter Milch unterschieden: 1. bis 2 Millionen, 2. von 2 bis 4 Millionen und 3. über 4 Millionen. Die Tiere waren klinisch gesund. 7 Tage lang wurden allen Tieren *per os* 12 mg je kg der Lebensmasse NAC bei der Abendmelkung verabreicht. Die Untersuchung von Milchleistung, Fett-, Eiweiß- und Laktosegehalt sowie die Anzahl der somatischen Zellen wurde an drei Zeitpunkten durchgeführt: 1. vor Beginn der Verabreichung von NAC, 2. nach einer Woche der Verabreichung sowie 3. nach einer Woche nach Einstellung der Verabreichung von NAC. Die Varianzanalyse wurde unter Anwendung des GLM-Verfahrens des SAS Programms durchgeführt. Die Verabreichung von NAC hat nach einer Woche eine wesentliche Minderung der Anzahl der somatischen Zellen verursacht sowie eine weitere Woche lang das Halten dieses Spiegels nachdem die Verabreichung von NAC in allen Gruppen eingestellt worden war. Es wurden keine wesentlichen Änderungen des Gehaltes der Milchbestandteile beobachtet.

Eine wesentliche Minderung der Anzahl der somatischen Zellen zeugt von einer deutlichen Verbesserung der Gesundheit der Milchdrüse der Ziegen. Wahrscheinlich wurden die Oxidationsprozesse gehemmt und die Abwehrkräfte des Organismus sind gestiegen. Der Zusatz von NAC könnte zur Verbesserung der Gesundheit der Milchdrüsen von Ziegen führen.

Schlüsselwörter: N-acetylcysteine, somatische Zellen, Ziegenmilch

Introduction

Mastitis causes large economic losses in goat herds. CONTRERAS et al. (2003) estimated that in herd with 30% cases of mastitis the losses due to decreased milk yield amounted to 36€ per goat, per lactation. The SCC is an indicator which is used for monitoring mammary gland health in cattle and goat breeding (HAENLEIN, 2002). However, in contrast to dairy cattle, the relationship between SCC and causes of subclinical mastitis in dairy goats is still controversial (ZENG et al., 1999). Some studies indicated that milk from healthy goat's udder, could show SCC as high as 5×10^6 (FAHR et al., 2001; PAAPE, 2000). SCC increases rapidly at the end of lactation while only 10% of goats have mammary infection at this period (CONTRERAS et al., 1999). But the bacterial infections of mammary gland always cause increase of SCC in goat milk (CONTRERAS et al., 1999). The treatment of goats with mastitis is connected with application of antibiotics. The presence of remains of antibiotics, pathogens and their toxins constitute risk for consumer (MITCHELL et al., 1998). Thus, searching for other methods helping to keep healthy glands seems to be very important. The increasing number of pathogens cause local inflammation and consequently increases of oxidative processes (JIALL et al., 2002). There are indications that reduced glutathione (GSH) and associated enzymes play an important role in cellular immunity (TOWNSEND et al., 2003). In the study, conducted on dairy cows, the connection between SCC and level of GSH was found (JÓŹWIK et al., 2004). GSH is a very important antioxidant (ŚLIWA-JÓŹWIK et al., 2002; URBAN et al., 1997) and plays a role in the detoxification of a variety of electrophilic compounds and peroxides via catalysis by glutathione S-transferases (GST) and glutathione peroxidases (GPx). The main precursor of GSH is N-acetylcysteine (NAC) which has a powerful effect on rising glutathione level in both human and animal organisms (URBAN et al., 1997). NAC is widely used in human treatment against chronic bronchitis (GRANDJEAN et al., 2000). NAC has an influence on macrophage and lymphocyte functions and thus on immunology system in mouse (PUERTO et al., 2002). The NAC seems to have an immunomodulating capacity, and could be a candidate for further evaluations in various types of acute or chronic infections and inflammations in which oxidative stress could occur (URBAN et al., 1997).

The aim of this preliminary study was to estimate an influence of N-acetylcysteine oral supplementation on the chemical composition of goat milk and somatic cell count.

Materials and methods

The study was conducted on 15 Polish White Improved goats in age 4-5 years, in the first trimester of pregnancy, in the last part of lactation (220-250 days). The average body weight of animals was about 55 kg. The goats were maintained in a loose barn at the Experimental Farm of the Institute of Genetics and Animal Breeding, Polish Academy of Sciences in Jastrzębiec. The animals were fed according to INRA guidelines. The goats were machine milked twice a day. They were under veterinary control and had no clinical signs of mastitis.

The animals were divided into three groups, 5 animals in each (n=5), according to SCC in their milk measured in the samples of milk from the morning milking just before the beginning of the experiment: 1st – SCC below 2×10^6, 2nd – SCC between 2 and 4×10^6, 3rd – SCC above 4×10^6.

All goats were given 12 mg/kg live body weight of NAC in capsules of company HEXAL®, per os, once a day during evening milking, for 7 days. The amount of NAC was determined based on indication of HEXAL® for human according to live body weight of does.
The milk samples were taken three times: just before NAC supplementation, on 8th day, and on 15th day from the start of experiment (1st, 2nd and 3rd time of sample collection). The third sample collection was done just before drying off goats. The milk yield and chemical composition of milk was determined using IR-spectrophotometer and SCC was found using apparatus based on fluorescence microscopy method.
Statistical analysis was done using GLM procedure of SAS Version 8e for Windows (SAS, SAS/STAT 1999-2001) using the model which included: time of sample collection and number of animal subgroup as the fixed interaction; fixed effect of age of goat; regression of chemical composition and lnSCC on milk yield.

Results and discussion

There were differences between Least Squares Means (LSM) values of SCC in three groups before start of the experiment at $p \leq 0.01$ (Table 1 – mean data in 1st column). After 7 days of NAC supplementation in all three groups the number of SCC decreased ($p \leq 0.01$, mean data in rows). There were no differences between groups in SCC in samples from second collection (mean data in 2nd column). The samples from third collection performed one week after the end of supplementation (data in 3rd column) showed small increase of SCC in all three groups. The difference in SCC was found only between first and third groups at $p \leq 0.01$. Furthermore, the number of somatic cells did not return to the level before NAC supplementation, even in the third group having the largest SCC.

Table 1
Effects of groups and times of sample collection on lnSCC – LSM and their standard errors (SE) (Einfluss von Gruppen und Entnahmereihenfolge der Milchproben auf LnSCC – LSM und ihre Standardabweichungen [SE])

Group	Time of sample collection					
	1		2		3	
	LSM	SE	LSM	SE	LSM	SE
1	7.49^{A} (A)	0.40	6.18^{B}	0.35	6.31^{B} (A)	0.38
2	8.55^{A} (B)	0.42	7.10^{B}	0.38	6.89^{B}	0.42
3	9.18^{A} (C)	0.34	7.08^{B}	0.35	7.54^{B} (B)	0.46

$^{A, B}$ = different letters within rows and columns (in brackets) indicate significant differences at $p<0.01$

The differences of milk yield (Table 2) between third and first, as well as third and second groups were found in first and second collections, while there were no differences between groups in third collection (data in columns). The milk yield in third collection in all three groups was lower than milk yield in first and second collections (data in rows) at $p \leq 0.01$.
Concerning chemical composition of milk (Table 3) there were no differences neither between groups in all three collections (data in columns), nor between samples collections in all three groups (data in rows).

Table 2
Effects of groups and times of collection on milk yield – LSM and their standard errors (SE)
(Einfluss von Gruppen und Entnahmereihenfolge der Milchproben auf Milchleistung – LSM und ihre Standardabweichungen [SE])

Group	Time of sample collection					
	1		2		3	
	LSM	SE	LSM	SE	LSM	SE
1	$1.80^{A\ (A)}$	0.16	$1.48^{A\ (a)}$	0.16	0.68^{B}	0.16
2	$1.76^{A\ (A)}$	0.17	$1.49^{A\ (a)}$	0.17	0.60^{B}	0.17
3	$1.11^{A\ (B)}$	0.16	$0.97^{A\ (b)}$	0.16	0.33^{B}	0.15

A, B = different letters within rows and columns (in brackets) indicate significant differences at $p<0.01$
a, b = different letters within columns (in brackets) indicate significant differences at $p<0.05$

Table 3
Effects of groups and times of collection on chemical composition of milk – LSM and their standard errors (SE)
(Einfluss von Gruppen und Entnahmereihenfolge der Milchproben auf chemische Milchzusammensetzung – LSM und ihre Standardabweichungen [SE])

Group	Trait	Time of sample collection					
		1		2		3	
		LSM	SE	LSM	SE	LSM	SE
1	% protein	3.56	0.24	3.20	0.21	3.33	0.23
	% fat	4.80	0.45	3.81	0.39	4.34	0.43
	% lactose	4.26	0.16	4.19	0.14	4.35	0.15
2	% protein	3.84	0.25	3.44	0.23	3.36	0.25
	% fat	4.24	0.46	3.31	0.42	3.27	0.46
	% lactose	4.09	0.16	4.19	0.15	4.29	0.16
3	% protein	4.08	0.21	3.45	0.21	3.91	0.28
	% fat	4.25	0.37	3.47	0.38	3.36	0.51
	% lactose	4.00	0.13	4.23	0.14	4.14	0.18

Physiological processes could explain permanent decreasing of milk yield in consecutive collections in the last stage of lactation – preparing udder for drying. Decrease of SCC from the beginning of experiment to the second collection of milk samples was caused probably by NAC supplementation.

Many authors paid attention to redox disturbance in inflammatory processes in last years (HE et al., 2003; SHI et al., 2004). Our earlier results (JÓŹWIK et al., 2004) showed that an organism defending itself from clinical mastitis increases the level of GSH. The NAC supplementation to goats producing milk having high amount of somatic cells caused significant decrease of their number. Nowadays, there is the lack of any information on the influence of NAC on processes occurring in healthy mammary gland as well as with clinical or subclinical mastitis. The study on humans indicated that oral supplementation of NAC might, directly or indirectly via metabolites, act as a scavenger of ROS (URBAN et al., 1997). N-acetylcysteine has a large impact on neutrophil, lymphocyte and macrophage functions (PUERTO et al., 2002; URBAN et al., 1997). These leucocytes constitute the part of somatic cells in milk and they play an important role in mammary gland immunity and are vital for protection against intramammary infection (BRADLEY, 2002). Therefore, it could be supposed that the decrease of SCC might mean that after NAC supplementation the oxidative processes were restrained and thus, the possibility of appearing of inflammation was limited. It might be suggested that the defence power of an organism was increased which could be connected with advantageous activity of –SH group of reduced glutathione. The –SH group of glutathione probably may easier access reactive oxidative species (ROS) than chemical groups of other antioxidants. Therefore, it could be used in convalescence of animals.

Summing-up, it could be underlined that the NAC supplementation had advantageous influence on SCC in milk of investigated goats. There were no negative changes in health of born offspring and their mothers. Obtained results indicate the need of conducting further, very detailed studies on influence of NAC on mammary gland health using microbiological and cytological analysis.

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