

# Influence of the presence of pathogenic bacteria on the relationship between *N*-acetylcysteine administration and the population of somatic cells in goat milk

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## Abstract

The objective of this study was to estimate the influence of per os *N*-acetylcysteine (NAC) administration on total somatic cell count (SCC) and their composition in goat milk depending on the presence of pathogenic bacteria. The study was conducted on 12 Polish White Improved (PWI) dairy goats, 3-7 years old, between 150<sup>th</sup> and 180<sup>th</sup> day of lactation. The milk samples (total 68) were taken from separate udder halves, three times during the study: just before NAC supplementation and on 8<sup>th</sup> and 15<sup>th</sup> day of the experiment. The total SCC and their composition was determined using flow cytometry. The milk samples were also cultured for presence of pathogenic bacteria which were revealed in the half of them. *N*-acetylcysteine administration influenced the total SCC, the content of total leukocytes and particular leukocyte subpopulation in total SCC both in milk from healthy and infected halves of an udder.

**Key words:** *N*-acetylcysteine, SCC, pathogenic bacteria, goat milk.

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## Introduction

*N*-acetylcysteine (NAC) is used in human medicine against bronchitis for its ability to break up mucus in the lungs [1]. Because NAC is the main precursor of reduced glutathione, which is one of the most important antioxidants, it seems that NAC has an immunomodulating capability [2]. The studies from last decade showed that NAC has probably great potential for preventing heart diseases, cancer and even aging itself in humans [3-5].

Moreover, NAC was shown to influence several macrophage, neutrophil and lymphocyte functions such as adherence, chemotaxis, phagocytosis, superoxide and natural killer activity in mice and humans [5, 6].

Neutrophils and macrophages are the predominating leucocytes in the somatic cells in goat milk [7]. The proportion of lymphocytes in goat milk may be as high as 10% or even 17% [8, 9]. It was shown in one study on dairy cows that the number of these subpopulations may increase in mastitis [10]. Our earlier study on dairy goats showed that the percentage of lymphocytes did not depend on the presence of bacteria and reached only 2.5%. The percentage of monocytes and eosinophils in goat milk ranged between 10-15% and 2.5-7%, respectively, depending on health status of the mammary gland (Bagnicka – data not published). Moreover our preliminary study showed significant influence of NAC diet supplementation on SCC

in goat milk [11] and on oxidative status of dairy goats [12]. *Per os* administration of NAC for one week caused the decrease and then stagnation of SCC in goat milk with low, medium and high somatic number [11], the decrease of MDA and GSH concentration in whole blood and the increase of vitamin C in blood serum [12]. Nevertheless the preliminary study was conducted without microbiological and cytological analysis of goat milk.

The objective of this study was to estimate the influence of NAC administration on total SCC and the composition of somatic cell population in goat milk depending on the presence of pathogenic bacteria.

## Material and methods

The study was conducted on 12 Polish White Improved (PWI) adult dairy goats at the age of 3-7 years. The animals were kept in the loose barn, and fed the diet composed of corn silage, wilted grass silage and concentrates, supplemented with mineral and vitamin mixture according to the INRA system [13]. Water was available at will. Goats were milked mechanically twice a day. All animals were checked for symptoms of clinical mastitis. The study was conducted between 150<sup>th</sup> and 180<sup>th</sup> day of lactation.

All goats had been given 12 mg of NAC/kg live body weight *per os* once a day during the evening milking, for first 7 days of the study. The HEXAL<sup>®</sup> capsules were used. The amount of NAC was determined based on indication of HEXAL<sup>®</sup> for humans and adjusted to the goat body weight. The milk samples (total 68) were taken from separate udder halves, during the routine morning milking, three times during the experiment: just before NAC was given for the first time, on 8<sup>th</sup> and on 15<sup>th</sup> day from the beginning of the study (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> sampling). The SCC was determined using Fossomatic 90 and the results were transformed to the natural logarithm scale for needs of statistical analysis.

The flow cytometry method was used to determine the content of particular subpopulations of the SCC. Leukocytes were identified by immunophenotyping, with species-specific monoclonal anti-CD45 antibody, labelled with fluorescein. The total number of 8000 milk cells was randomly selected to determine the proportion of leukocytes as well as proportions of individual leukocyte subpopulations (eosinophils, neutrophils, monocytes and lymphocytes) in goat milk.

Each milk sample was cultured on 5% sheep blood enriched Columbia agar and MacConkey agar (bioMérieux, France) for presence of pathogenic bacteria. Both media were inoculated with 100  $\mu$ l and 10  $\mu$ l of specimens. Plates were incubated at 37°C for 48 hours. Then bacteria were identified and quantified by standard laboratory techniques [14]. The API 20 E, API Staph and API 20 STREP tests (bioMérieux, France) were used to determine the biochemical activity of isolated bacteria. Staphylococci

were detected using a tube test with rabbit plasma and then Staphylococcus aureus strains were identified using Slidex Staph-Kit (bioMérieux, France). The presence of Mycoplasma spp. was not determined. For statistical analysis the samples were divided into two groups according to the lack or presence of bacteria (milk from non-infected and infected udders, respectively).

Statistical analysis was performed using GLM procedure of SAS Version 9.1 for Windows (SAS, SAS/STAT 2002-2003). The model including time of sampling and presence of bacteria as the fixed interaction and random effect of animal was applied. The analysis of variance of the total SCC and proportion of total leucocytes as well as individual leukocyte subpopulation in the total SCC was performed.

All procedures involving animals were performed in accordance with the Guiding Principles for the Care and Use of Research Animals and were approved by the Local Ethics Commission (Permission No. 48/2005).

## Results

The goats had no clinical signs of mastitis. The half of milk samples contained pathogenic bacteria. They were mainly *Staphylococcus* spp. and *Streptococcus* spp. There was no relationship between the age of goats and the investigated features. Moreover the interaction between the time of sampling and the presence of pathogens had no impact on milk yield (data not presented). The influence of the presence of bacteria on total SCC and leukocytes in subsequent samplings is presented in table 1 and 2, respectively. In turn the impacts of the presence of bacteria on the proportion of individual leukocyte subpopulations (namely eosinophils, neutrophils, monocytes and lymphocytes) in subsequent samplings are presented in table 3-6, respectively.

## Discussion

Initially the SCC was similar in milk samples from non-infected and infected udders. Nevertheless at 2<sup>nd</sup> and 3<sup>rd</sup> sampling SCC was lower ( $p \leq 0.05$ ) in milk samples from non-infected udders. In infected udders the SCC was the same at the start of experiment and after one week of NAC administration. The SCC in both groups increased ( $p \leq 0.01$ ) after the termination of NAC administration. This study confirmed our previous results [11, 12]. The SCC in milk from infected udders remained unchanged after NAC administration. It may mean that NAC promoted immune reaction of the body and aided in inhibiting the proliferation of pathogenic bacteria or inhibited the activity of chemokines for SCC and thus the influx of SCC to the milk remained unchanged. Once NAC administration was terminated the influx of SCC to the milk rose, probably due to increased activity of leukocyte chemokines. The NAC

**Table 1.** Relationship (LSMEAN,  $\pm$ SE) between the presence of bacteria and total SCC in subsequent samplings

Bacteria in milk	Sampling		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Absent	6.84 $\pm$ 0.19 a	6.16 $\pm$ 0.26 (a) A, b	7.20 $\pm$ 0.20 (a) B
Present	7.03 $\pm$ 0.27	6.84 $\pm$ 0.16 (b) A	7.82 $\pm$ 0.19 (b) B

A, B – different letters in rows indicate statistical significance at  $p \leq 0.01$   
a, b – different letters in rows and columns (in brackets) indicate statistical significance at  $p \leq 0.05$

**Table 3.** Relationship (LSMEAN,  $\pm$ SE) between the presence of bacteria and the proportion of eosinophils in total SCC in subsequent samplings

Bacteria in milk	Sampling		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Absent	13.46 $\pm$ 2.17 A	3.61 $\pm$ 2.98 B, a	11.06 $\pm$ 2.23 b
Present	12.25 $\pm$ 3.13 A	2.37 $\pm$ 1.84 B	13.57 $\pm$ 2.20 A

A, B – different letters in rows indicate statistical significance at  $p \leq 0.01$   
a, b – different letters in rows and columns (in brackets) indicate statistical significance at  $p \leq 0.05$

**Table 5.** Relationship (LSMEAN,  $\pm$ SE) between the presence of bacteria and the proportion of monocytes in total SCC in subsequent samplings

Bacteria in milk	Sampling		
	1 <sup>st</sup>	8 <sup>th</sup>	15 <sup>th</sup>
Absent	7.52 $\pm$ 3.10 a	17.38 $\pm$ 4.27 b	16.10 $\pm$ 3.19 a
Present	3.77 $\pm$ 4.84 A, a	22.56 $\pm$ 2.64 B	17.81 $\pm$ 3.15 b

A, B – different letters in rows indicate statistical significance at  $p \leq 0.01$   
a, b – different letters in rows and columns (in brackets) indicate statistical significance at  $p \leq 0.05$

administration had no influence on leukocyte content in total SCC both in milk from non-infected and infected udders. However, termination of NAC administration resulted in the increase of leukocyte proportion in total SCC, comparing with exfoliated epithelial cells. It is very likely that the more intensive influx of leukocytes from bloodstream to the milk in response to the re-proliferation of bacteria was responsible for this reaction. Moreover, the NAC administration had the influence on the proportion of individual leukocyte subpopulations in total SCC. On the other hand apart from the lymphocytes, proportions of other leukocyte subpopulations showed no difference between the milk from non-infected and infected udders. The proportion of eosinophils dropped dramatically after NAC administration and returned to baseline at the 3<sup>rd</sup> sampling. The proportion of monocytes also changed considerably. It

**Table 2.** Relationship (LSMEAN,  $\pm$ SE) between the presence of bacteria and the proportion of leucocytes in total SCC in subsequent samplings

Bacteria in milk	Sampling		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Absent	54.57 $\pm$ 5.01	42.30 $\pm$ 6.90	56.58 $\pm$ 5.17
Present	40.40 $\pm$ 7.25 A	49.76 $\pm$ 4.27 a	63.48 $\pm$ 5.10 B, b

A, B – different letters in rows indicate statistical significance at  $p \leq 0.01$   
a, b – different letters in rows and columns (in brackets) indicate statistical significance at  $p \leq 0.05$

**Table 4.** Relationship (LSMEAN,  $\pm$ SE) between the presence of bacteria and the proportion of neutrophils in total SCC in subsequent samplings

Bacteria in milk	Sampling		
	1 <sup>st</sup>	8 <sup>th</sup>	15 <sup>th</sup>
Absent	30.52 $\pm$ 4.18 a	16.38 $\pm$ 5.77 b	25.41 $\pm$ 4.31
Present	23.84 $\pm$ 6.05	17.62 $\pm$ 3.56 a	27.96 $\pm$ 4.26 b

A, B – different letters in rows indicate statistical significance at  $p \leq 0.01$   
a, b – different letters in rows and columns (in brackets) indicate statistical significance at  $p \leq 0.05$

**Table 6.** Relationship (LSMEAN,  $\pm$ SE) between the presence of bacteria and the proportion of lymphocytes in total SCC in subsequent samplings

Bacteria in milk	Sampling		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Absent	2.28 $\pm$ 0.31 (a) A, a	1.26 $\pm$ 0.42 b	0.96 $\pm$ 0.32 B
Present	0.91 $\pm$ 0.44 (b)	1.67 $\pm$ 0.26 a	0.65 $\pm$ 0.31 b

A, B – different letters in rows indicate statistical significance at  $p < 0.01$   
a, b – different letters in rows and columns (in brackets) indicate statistical significance at  $p \leq 0.05$

increased following NAC administration and remained on the high level for a week after termination of NAC administration. Proportions of neutrophils and lymphocytes were not subject to so significant fluctuations but NAC administration diminished the proportion of neutrophils in milk from non-infected udders whereas termination of NAC administration led to the increase of this figure in milk from infected udders. Prior to NAC administration proportion of lymphocytes was higher ( $p < 0.05$ ) in milk from non-infected than infected udders. After NAC administration proportion of lymphocytes in milk from non-infected udders fell ( $p < 0.05$ ) so much that proportion of lymphocytes in milk from infected udders became higher at the 2<sup>nd</sup> sampling ( $p < 0.05$ ). Despite the termination of NAC administration the proportion of lymphocytes in milk from non-infected udders kept on falling. Variations in

lymphocyte content in milk from infected udders at subsequent samplings were not statistically significant but substantial standard errors indicate high variation of this feature. Strong influence of NAC on macrophage and lymphocyte function in mice as well as on neutrophil and lymphocyte function in healthy humans was demonstrated in several studies [2, 5, 6]. It is consistent with results of our study with respect to lymphocytes as well as monocytes which, in fact are immature macrophages.

It can be concluded that N-acetylcysteine administration affected the total SCC, total leukocytes and individual leukocyte subpopulations both in milk from non-infected and infected udders. As NAC has an influence on the composition and function of somatic cells in milk (mainly phagocytosis of neutrophils and natural killer activity of lymphocytes) it can modulate the immune system and improve the health status of an udder. Although NAC has no direct bactericidal effect it can assist in inhibition of pathogenic bacteria growth by promoting the activity of the local immune system.

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