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The effect of n-3 unsaturated fatty acids addition on somatic and microorganism cell count in goats' milk

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Introduction

Park (2009) stated that goat milk has high nutritional value and a large number of bioactive ingredients. Yadav (2016) emphasized that goats' milk is more easily digestible comparing to cows' milk, mainly because of its higher β-casein content, smaller fat globule diameter and lack of agglutinin. These goat milk properties allow us to categorize it as 'functional foods'. On the other hand, accordingly to Ney (1991) goat's milk contains a high value of saturated fats which consumption could be considered a risk factor for the occurrence of cardiovascular disease. Furthermore, accordingly to some researches (Daviglus et al., 1997; Albert et al., 1998) goats' milk are deficient with polyunsaturated fatty acids (PUFA), which are correlated with a decrease of defined risk factor. The supplementation of ruminants' ration with different types of protected fats could modify quantity and quality of milk making it more suitable for human consumption (Grummer, 1991; Ashes et al., 2000; Gagliostro and Chilliard, 1992).

The essentiality of polyunsaturated fatty acids (PUFA) is reflected in the fact that they are incorporated into cell membrane phospholipids. The supply of PUFA, due to richness of the membranes of the immune cells with PUFA, has a significant effect on the immune system. Unbalanced supply of FA (inadequate ratio between n-3 and n-6 FA) could result in occurrence of inflammatory processes. Furthermore, the addition of n-3 FA may have anti-inflammatory effect. This effect has already been used in human medicine for treatment of chronic inflammatory diseases. Since, inflammatory processes can also occur in goats resulting in, possibly, increase of somatic cells in milk, the short-term addition of relatively large amounts of n-3 FA could have a beneficial effect on inflammatory processes, and consequently on somatic cell count.

Therefore, the aim of this study was to determine whether short-term addition of a large amount of different n-3 FA (eicosapentaenoic, α-linoleic, and docosahexaenoic) has an effect on somatic and microorganism cell count in goats' milk.

Furthermore, the duration of this effect after the supplementation was analyzed.

Material and methods

Experimental design.

The study was performed on 62 Alpine and 28 Saanen goats breed on an indoor dairy farm. The goats included in study were in 30 to 140 days of lactation, weighted in average 51 kg (±6 kg), with weaned goatling. Milking was machined and performed twice a day (in the morning from 5.30 till 6.30 and in the evening from 17.30 till 18.30. The goats had hay twice a day *ad libitum* as basic meal, and feed mixture (50% ground corn grain, 30% dried beet noodles and 20% wheat bran) at the time of milking. In accordance to the addition of supplement, the study was divided in three periods: before supplementation that lasted 9 days (BS); supplementation period that lasted 5 days (S); and after supplementation that lasted 50 days (AS). Before supplementation period (BS) presented the goats' adoption to the feed mixture and people involved in milking. After adoption, accordingly to supplemented n-3 PUFA, the goats were randomly allocated into 4 groups (Table 1). During the supplementation period (S), n-3 PUFA were supplemented through a tube which was introduced into the animals' oesophagus every morning during milking in amount of 20 g/day. During the BS, S and first five days of the AS period, milk sampling (70 ml) from each goat was performed daily at each milking, while from the 6th to the 50th day of the AS period, milk was sampled every fifth day. The 0.2 ml of azidiol - NaN_3-based preservative at a concentration of 0.02% was added with the addition of chloramphenicol to "stabilize" the microorganisms in all samples. Samples were placed on cold and delivered once a day to the Dairy Laboratory of the Department of Zootechnics, Biotechnical Faculty, Ljubljana.

Table 1. Group of animals accordingly to the supplementation

Group	n-3 unsaturated fatty acid	Supplement
G-1	eicosapentaenoic acid (EPA)	oil produced by Pronova Biocare, Norway containing 94.93 wt% of EPA
G-2	α-linoleic acid (ALA)	linseed oil produced by A.C.E.F. Lex containing 57.84 wt% of α-linoleic acid; 19.10 wt% of oleic acid and 14.35 wt% of linoleic acid
G-3	docosahexaenoic acid (DHA)	oil produced by Nippon Chemical Feed Co containing 74.75 wt% of DHA, 5.84 wt% of EPA and 2.05 wt% DPA
G-4	control group	no supplement was added

The number of somatic cells in milk were determined using Fossomatic 5000 device, acting in accordance with ISO 13366-3 (1997). The device operates on the principle of automatic epifluorescence technique, that is, the principle of flow cytometry. Coloured somatic cells are passed through the cytometric cell one by one with a fluorescent dye, and the instrument counts them automatically. The total number of microorganisms was determined with a BactoScan 8000 device, type 27000 (Foss Electric). An automatic chemical and physical preparation of the sample was carried out before analysis, which eliminates the possibility of the effect of other constituents of milk (somatic cells, proteins) on the result. The microorganism cells were stained with Acridine orange and illuminated with xenon lamp light. Because of this, coloured bacterial cells emit light pulses that are measured with four photodetectors that are in the microscope. The electrical impulse is converted to bacterial cell count.

Statistical analysis.

The SAS/STAT package was used (SAS Institute Inc., 2000) for the preparation, logical control and statistical analysis of data. The effects of the experimental group (G-1, G-2, G-3, and G-4) and experimental periods (BS, S, and AS) on somatic and microorganism cell count in milk at milking were tested using GLM procedure with a nested design. Duncan's Multiple Range Test was used for testing the differences between the groups.

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Results and discussion

The number of somatic cells (SC) is one of the most variable traits in milk. In this study, an average number of 1,208,000 SC in ml of milk, with SD = ±2,064,000 and KV of 170.8% was found. Furthermore, values ranged from 13,000 to 24,312,000 SC in ml of milk. Transformation into logarithmic value enabled analysis of variability and determination of effects. The somatic cell count at milking (log10) in regard to experimental period and supplementation group is presented in the Table 2.

Table 2. The somatic cell count in milk at milking (log_{10}) in regard to experimental period and supplementation group.

Experimental period	Supplementation group			
	G-1	G-2	G-3	G-4
Before supplementation (BS)	2.69 ^{a}	2.68 ^{a}	2.62 ^{a}	2.54 ^{a}
Supplementation (S)	2.82 ^{a}	2.72 ^{a}	2.80 ^{a}	2.99 ^{a}
After supplementation (AS)	2.83 ^{a}	2.77 ^{b}	2.88 ^{a}	2.99 ^{a}

[1] Values within the same row marked with different letter differ statistically significant ($p < 0.05$).

The nonpathological factors affecting the number of SCs in goat's milk in commercial breeds are quite different from those in cow's milk since goats breeding are mostly seasonal, when all animals are in the same lactation period, which is not generally the case with cows. This allows for easier procedures for providing physiological SC counts in cow's milk throughout the year, as cows are on average herding at all stages of lactation at all times. Aleandri et al. (1994) stated that the estrus has a strong effect on the number of SCs, as they have found an increase in the number of SCs at the time when goats were in estrus. They say, however, that this may be associated with stage of lactation, because in this period goats in the last period of lactation. Haenlein (2002) reported that there is a varying number of SCs in milk in late-lactation depending on the amount of milk being milked. Furthermore, there are fewer in the beginning and middle of the lactation than towards the end of the lactation, when their numbers increase significantly (Haenlein, 2002). According to parity, it is noted that consecutive lactation also increases the number of SC in milk (Rota et al., 1993). Also, stress can be caused by feed (especially large amounts of added concentrate at a time), which leads to acidosis and an increase in SC. Furthermore, other inadequate conditions, such as barn climate, temperature, and animal handling also have an effect on SC increases.

The udder inflammation (mastitis), in small ruminants, is usually associated with a large number of SC. In the research of the effect of PUFA supplementation on SC, Košmelj et al. (2001) determined the effect of the addition of α-linoleic acid, which was reflected in the decrease in somatic cell counts at the time of addition and also 4 weeks after it. Accordingly, to Adam et al. (1986) α-linoleic acid (ALA) incorporates in phospholipids 5 hours after supplementation while the other two (eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA) only after a few days after addition. So perhaps the other two would have the same effect, especially DHA, which is expected to have a progressive anti-inflammatory effect in terms of the number of double bonds and the length of the molecule (Rosenwasser, 1998; James et al., 2000; DeCaterina et al., 2000).

The microorganism cell count at milking (log10) in regard to experimental period and supplementation group is presented in the Table 3. In terms of the number of microorganisms in milk, it is known that it depends to a great extent on the hygiene of milking, that is, on staff, on animals, as well as on equipment usage, hygienic maintenance and cleaning of equipment, and of course on udder health and the presence of mastitis. Thus, in this study, hygiene and cleaning improved significantly shortly after the start of the study, thus reducing the number of microorganisms in milk, while mastitis was absent throughout the study. Haenlein (2002) stated that the number of bacteria in milk highly depends of milking methods. Also, he determined that the number of bacteria was positively correlated (r = 0.14) with the number of SCs.

Table 3. The microorganism cell count in milk at milking (log_{10}) in regard to experimental period and supplementation group.

Experimental period	Supplementation group			
	G-1	G-2	G-3	G-4
Before supplementation (BS)	2.64 ^{a}	2.62 ^{a}	2.47^{a}	2.58 ^{a}
Supplementation (S)	2.44 ^{a}	2.44 ^{a}	2.48 ^{a}	2.62 ^{b}
After supplementation (AS)	2.35 ^{a}	2.34 ^{a}	2.34 ^{a}	2.48 ^{a}

[1] Values within the same row marked with different letter differ statistically significant ($p < 0.05$).

Given the known facts from the above data, it can be seen that the oscillation of SC in goats is subject to many effects. In past decades, researchers have given n-3 FA to ruminants in order to improve FA milk and meat composition, but they have not monitored the effect this has on somatic cell counts. This study clearly shows that the addition of α-linoleic FA had a relatively long effect on the reduction of somatic cells in milk. This could be explained by the fact that the addition leads to more appropriate ratio of n-3 and n-6 PUFA, which was not provided with feed alone.

Conclusion

The short-term supplementation of dairy goats with a large amount of different n-3 fatty acids (α-linoleic, eicosapentaenoic and docosahexaenoic, PUFA) effected both the somatic cell and microorganism cell count in milk at milking. The addition of supplements induced the reduction of somatic cells during the supplementation in all experimental groups, compared to the control one. The lowest values were in the group to which α-linoleic acid was added, although there were no statistically significant differences. This trend maintained even in the period after supplementation until the end of the study. Furthermore, during the supplementation of PUFA, the values of microorganisms in milk in the supplementation groups were statistically significantly lower ($p < 0.05$) compared to the control group. In the period after supplementation, the values of the microorganisms in the experimental groups remained at a lower level than in the control group.

To conclude, the addition of unsaturated fatty acids has a positive effect on the number of somatic cells and the number of microorganism cells in milk. Furthermore, further research is needed to determine the exact formulation of the addition of unsaturated fatty acids.