University of Banjaluka, Faculty of Agriculture

Agro-knowledge Journal

Variation in Milk Composition of Dairy Goats due to N-3 Unsaturated Fatty Acids Supplementation

Vesna Gantner[1], Mirna Gavran[1], Maja Gregić[1], Božo Važić[3],
Ranko Gantner[1], Klemen Potočnik[2]

[1]*University of Josip Juraj Strossmayer in Osijek, Faculty of Agrobiotechnology, Croatia*
[2]*University of Ljubljana, Biotechnical Faculty, Domžale, Slovenia*
[3]*University of Banja Luka, Faculty of Agriculture, Republic of Srpska, BiH*

Abstract

The objective of this research was to determine the effect of addition of n-3 unsaturated fatty acids (α-linoleic, eicosapentaenoic and docosahexaenoic) in dairy goats' ration on milk composition (fat and protein content). Also, the persistence of the effect after supplementation was analysed. The research was conducted on dairy goats (Alpine and Saanen) bred at an indoor farm. Regarding the experimental period, the milk sampling at milking (morning and evening) was performed in the period before supplementation (BS), during supplementation (S), and after supplementation (AS). According to the added supplement, animals were randomly allocated into a group (G-4) with no added supplement and three test groups (G-1; G-2; G-3) where a supplement containing n-3 unsaturated fatty acid was added over a period of five days. The results of this research indicate that the addition of PUFA in goats' ration changes the milk composition. The supplementation of α-linoleic resulted in increase of both milk fat and protein content. Furthermore, the addition of eicosapentaenoic and docosahexaenoic resulted in decrease of fat but increase of protein content in milk. The observed trends also persisted after the supplementation period.

Key words: dairy goats, milk composition, n-3 unsaturated fatty acids supplementation.

Introduction

The polyunsaturated fatty acids (PUFA) have been known as essential for sixty or seventy years. Clear symptoms of essential fatty acid (FA) deficiency in adults were difficult to determine, but in infants fed with fat-free food the n-6 FA deficiency was determined. The importance of polyunsaturated fatty acids (PUFA) is reflected in that they are incorporated into phospholipids of cell membranes. There, polyunsaturated fatty acids act as building blocks of cell membranes as well as precursors for the synthesis of tissue (eicosanoid) hormones. Furthermore, fatty acids composition of cell membranes depends on the diet, i.e. the uptake of eicosanoid n-3 and n-6 fatty acids and the uptake of their precursors, that is, linoleic and α-linoleic acids. Therefore, unbalanced diet could result in a lack of essential fatty acids and / or an unfavourable ratio of n-3 and n-6 FAs. The significant effect of the supply of PUFA on the immune system was determined in several researches (Alexander, 1998; Nettelton; 1995; Reily et al., 1991).

This is explained by the fact that the membranes of the immune cells contain most eicosanoids and are markedly rich in PUFA. In a case that the supply of the FA is too much n-6 FA side, then this is reflected in similar lack of n-3 FA and consequently leads to inflammation. In that case, the addition of n-3 FA may have the opposite effect. This anti-inflammatory effect of n-3 FA is used in human medicine by modulating various chronic inflammatory diseases and atherosclerosis and malignancies with the addition of n-3 FA. According to Venkatraman et al. (1992) and Peterson et al. (1999), the anti-inflammatory effect implies large doses of all FA in a meal (4 to 5%). Furthermore, Fisher et al. (1990) determined that the prolonged increased n-3 FA uptake has immunosuppressive effect, and, among other things, inhibits bactericidal action of macrophages. Therefore, Palombo (Palombo et al., 1996, 1999) added large doses of n-3 FA for a short time first in large animal studies and then in clinical trials briefly, thus reducing the immune depressive effect of FA on immune cells while maintaining anti-inflammatory action of n-3 species eicosanoids.

Excessive inflammatory processes can also occur in animals. In dairy animals, one possible consequence of excessive inflammatory responses could be the appearance of somatic cells in milk. Since the short-term addition of relatively large amounts of n-3 FA in humans has proven to have a beneficial effect on inflammatory processes, a similar effect is expected in animals. Furthermore, the beneficial effect of n-3 FA addition could also affect milk production.

Therefore, the aim of this study was to determine the effect of short-term addition of a large amount of three different n-3 FA (α-linoleic, eicosapentaenoic

and docosahexaenoic) on composition of goats' milk as well as the effect persistence.

Material and Methods

Experimental design

The study was conducted on an indoor dairy farm where 62 Alpine and 28 Saanen goats were bred. According to lactation stage, the goats were from 4 to 20 weeks after parturition, with average body weight of 51 kg (±6 kg), and all kids were weaned. Furthermore, the goats were machine-milked twice a day, during the morning at 6.00 (± 30 min) and during the evening at 18.00 (± 30 min). The basic diet, hay, was given to animals twice a day, ad libitum. The goats also had a concentrate mixture (50% ground corn grain, 30% dried beet pulp and 20% wheat bran) at the milking parlour, which they consumed at each milking. In regards with supplementation, the experiment was divided into three periods: BS – before supplementation (in duration of 9 days); S – supplementation (5 days long); and AS – after supplementation (in duration of 50 days). Before supplementation (BS) presented the period of adoption of animals to the working group of people who participated in each milking. After that, according to the added n-3 unsaturated fatty acid, the animals 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 the amount of 20 g/day. During the BS, S and first five days of the AS period, milk sampling (70 ml) was performed every day at each milking from each animal, while from the 6th to the 50th day of the AS period, milk was sampled every fifth day.

Tab. 1. Groups of animals according to 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% of DPA
G-4	control group	no supplement was added

In all samples 0.2 ml azidiol - NaN3-based preservative at a concentration of 0.02% was added with the addition of chloramphenicol to

"stabilize" the microorganisms. Samples were placed on cold and delivered once a day to the Dairy Laboratory of the Department of Zootechnics, Biotechnical Faculty, Ljubljana.

The MilkoScan 133 B (FossElectric Hillerød, Denmark) according to IDF 141B: 1996 was used for the analysis of chemical composition analysis: % fat and % protein in sample. The instrument operates on the principle of infrared spectrometry, and was previously calibrated according to the Gerber method for fat, and the Kjeldahl method for protein. For calibration, 10 specific goat milk samples were used and the result was expressed as a percentage (g / 100 g).

Statistical analysis

To prepare logical control of data and statistical analysis, the SAS/STAT package was used (SAS Institute Inc., 2000). The effects of the experimental group (G-1, G-2, G-3, and G-4) and experimental periods (BS, S, and AS) on milk composition (fat and protein) at milking were tested using the GLM procedure with a nested design. The Duncan's Multiple Range Test was used to test the differences between the groups.

Results and Discussion

The average value of milk fat during the experiment was 2.98% (SD = ±0.60, CV = 20.14%). The composition of milk fat at milking according to the experimental period and supplementation group is presented in Table 2. Regarding the experimental period, the statistically significant increase ($p < 0.001$) of fat composition was determined during and after the supplementation of α-linoleic acid (ALA, group G-2) compared with fat composition in the period before supplementation.

Tab. 2. The composition of milk fat at milking (%) according to the experimental period and supplementation group

Experimental period	Supplementation group			
	G-1	G-2	G-3	G-4
Before supplementation (BS)	3.05[a]	3.15[a]	3.00[a]	2.99[a]
Supplementation (S)	2.65[a]	3.40[b]	2.52[a]	2.91[a]
After supplementation (AS)	2.84[a]	3.30[b]	2.77[a]	3.06[a]

*Values within the same row marked with a different letter differ statistically significantly ($p < 0.001$)

Furthermore, the addition of eicosapentaenoic acid (EPA, G-1) and docosahexaenoic acid (DHA, G-3) resulted in decrease of fat composition during the supplementation period (3.05% to 2.65%; 3.00% to 2.52%), with slight increase in the period after supplementation (2.65% to 2.84%, 2.52% to 2.77%).

The negative effect of addition of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on formation of fat in milk was determined in other researches (Chilliard, 1993; Kitessa et al., 2001; Ashes et al., 2000). On the other hand, Hermansen (1989) indicated that greater energy intake received as fat could lead to greater production of milk fat. Also, Sanz Sampelayo et al. (2004) observed that dietary addition of a PUFA produced an increase in milk fat which persisted after the dietary supplement was withdrawn. Neetika et al. (2019) reported no significant changes in milk fat when adding the linseed and chia oil with the highest value of milk fat in group of goats supplemented with linseed oil. Furthermore, Chilliard et al. (2001) found that the percentage of palmitic, stearic and oleic fatty acids in milk fat decreases when animals were supplemented with unsaturated fatty acids. Boeckaert at el. (2007) and Zhao et al. (2016) reported that the long-chain PUFAs (EPA and DHA) disrupt the bio-hydrogenation process at the ruminal level inhibiting ruminal conversion to stearic acid. Also, Neetika et al. (2019) determined that the addition of linseed oil improved proportion of α-linolenic, eicosapentaenoic and docosahexaenoic acid in goats' milk fat.

Tab. 3. The composition of milk protein at milking (%) according to the experimental period and supplementation group

Experimental period	Supplementation group			
	G-1	G-2	G-3	G-4
Before supplementation (BS)	2.93 [a]	3.12 [a]	2.98 [a]	3.01 [a]
Supplementation (S)	3.01 [a]	3.21 [b]	3.01 [a]	3.06 [a]
After supplementation (AS)	3.15 [a]	3.28 [a]	3.29 [a]	3.07 [b]

*Values within the same row marked with a different letter differ statistically significantly. ($p < 0.001$)

Protein content in milk during the experiment averaged at 3.19%, (SD = ±0.55, CV = 17.2). There were no significant differences in protein content between the supplementation groups before the addition of supplement (Table 3). At the supplementation period, the percentage of protein increased, especially in group G-2 where α-linoleic acid (ALA) was added. Furthermore, in the supplementation groups, protein contents tend to increase even after the of the supplementation.

The majority of researchers have found a decreased milk protein concentration in cows, which is thought to be due to the cytotoxic effect of PUFA on microbes in the rumen. This results in reduced protein synthesis. Ashes et al. (2000) concluded that the addition of voluminous feed binds PUFA to the surface and thus reduces the cytotoxic effect of the so-called detergent effect. Kitessa et al. (2001), however, similarly to this research, found an increase in milk protein in goats from 3.20% in the control group to 3.55% in the group fed with unprotected PUFA and 3.33% in the group fed with protected PUFA. This effect may be due to the feeding of dehydrated alfalfa pellets and oats (Kitessa et al. 2001) or dry hay and the addition of feed in our case, or the goat's microflora may not be so sensitive to the cytotoxic effect of PUFA. Garnsworthy (1996) indicated that the introduction of a protected fat into the ration may lead to a decrease in the milk protein content and yield.

This decrease could be explained by reduced food intake, which results in lower microbial protein synthesis in the rumen and consequently in reduced synthesis of protein in the mammary gland. Sanz Sampelayo et al. (2004) reported that the positive effect of dietary addition of a PUFA on milk fat and protein yields persisted also after supplementation. Neetika et al. (2019) determined higher values (but not significantly different) of protein content in milk of goats supplemented with Linseed and Chia oil. They determined the highest value of protein in goats supplemented with Chia oil. Finally, they concluded that the addition of plant oils alters, but not significantly, milk yield or composition but significantly affected fatty acid profile.

Conclusion

The results of this research indicate that the addition of n-3 unsaturated fatty acid (eicosapentaenoic acid, EPA; α-linoleic acid, ALA; and docosahexaenoic acid, DHA) in goats' ration changes the milk composition. In goats supplemented with ALA, milk fat increased during the supplementation period, while the addition of EPA and DHA resulted in decrease of fat composition. The observed trends also persisted after supplementation. Furthermore, in all supplementation groups, the milk protein increased when supplementing, with highest increase in animals with added ALA. Also, protein content tends to increase even after the withdrawal of the supplement.

References

Alexander, J.W. (1998). Immunonutrition: the role of ω-3 fatty acids. *Nutrition*, *14*(7-8), 627-633. doi: 10.1016/s0899-9007(98)00004-5

Ashes, J.R., Gulati, S.K., Kitessa, S.M., Fleck, E. & Scott, T.W. (2000). Utilisation of rumen protected n-3 fatty acids by rumen. In: Garnsworthy P.C., Wiseman J.(Eds.), *Recent advances in animal nutrition*, 129-140. Nottingham: Nottingham University Press.

Boeckaert, C., Fievez, V., Van Hecke, D., Verstraete, W. & Boon, N. (2007). Changes in rumen biohydrogenation intermediates and ciliate protozoa diversity after algae supplementation to dairy cattle. *European Journal of Lipid Science and Technology*, *109*(8), 767–777. doi: 10.1002/ejlt.200700052

Chilliard, Y. (1993). Dietary Fat and Adipose Tissue Metabolism in Ruminants, Pigs, and Rodents: A Review. *Journal of Dairy Science*, *76*(12), 3897–3931. doi:10.3168/jds.s0022-0302(93)77730-9

Chilliard, Y., Ferlay, A. & Doreau, M. (2001). Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livestock Production Science*, *70*(1-2), 31–48. doi:10.1016/s0301-6226(01)00196-8

Fisher, M., Levine, P.H., Weiner, B.H., Johnson, M.H., Doyle, E.M., Ellis, P.A. & Hoogasian, J.J. (1990). Dietary n-3 fatty acid supplementation reduces superoxide production and chemiluminescence in a monocyte-enriched preparation of leukocytes. *The American Journal of Clinical Nutrition*, *51*(5), 804–808. doi:10.1093/ajcn/51.5.804

Hermansen, J. E. (1989). Feed intake, milk yield and milk composition by replacing unprotected fat by Ca-soaps for dairy cows. *Animal Feed Science and Technology*, *22*(3), 193–202. doi: 10.1016/0377-8401(89)90061-8

Kitessa, S.M., Gulati, S.K., Ashes, J.R., Fleck, E., Scott, T.W. & Nichols, P.D. (2001). Utilisation of fish oil in ruminants. *Animal Feed Science and Technology*, *89*(3-4), 201–208. doi:10.1016/s0377-8401(00)00232-7

Neetika, J., Hundal, S., Wadhwa, M., Kaswan, S. & Sharma, A. (2019). Potential of chia oil to enrich goats' milk with omega-3 fatty acids in comparison to linseed oil under tropical climate. *Indian Journal of Animal Sciences*, *89*(3), 269–275.

Nettelton, J.A. (1995). Omega-3s and imune/inflammatory responses. Nettelton J.A. (Ed.), *Omega-3 fatty acids and health* (pp. 187-248). New York: Chapman & Hall.

Palombo, J.D., DeMichele, S.J., Boyce, P.J., Lyndon, E.E., Liu, J.W. & Huang, J.S. (1999). Effect of short–term enteral feeding with eicosapentaenoic and γ-linoleic acid on alveolar macrophage eicosanoid synthesis and bactericidal function in rats. Crit. Care Med., 27: 1908-1915.

Palombo, J.D., DeMichele, S.J., Boyce, P.J., Lydon, E.E., Liu, J.W., Huang, Y.S. & Bistrian, B. R. (1999). Effect of short-term enteral feeding with eicosapentaenoic and γ-linolenic acids on alveolar macrophage eicosanoid synthesis and bactericidal function in rats. *Critical Care Medicine*, *27*(9), 1908–1915. doi:10.1097/00003246-199909000-00032

Peterson, L. D., Thies, F. & Calder, P. C. (1999). Dose-dependent effects of dietary γ-linolenic acid on rat spleen lymphocyte functions. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *61*(1), 19–24. doi:10.1054/plef.1999.0067

Reilly, P.M., Schiller, H.J. & Bulkley, G.B. (1991). Reactive oxygen metabolites in shock. Wilmore, D.W., Brennan, M., Harken, A., Holkroft, J., Meakins, J.C. (eds.), *Care of the surgical patient* (pp. 1-27). New York: Scientific American Medicine.

Sanz Sampelayo, M. R., Martín Alonso, J. J., Pérez, L., Gil Extremera, F. & Boza, J. (2004). Dietary Supplements for Lactating Goats by Polyunsaturated Fatty Acid-Rich Protected Fat. Effects After Supplement Withdrawal. *Journal of Dairy Science*, *87*(6), 1796–1802. doi:10.3168/jds.s0022-0302(04)73335-4

SAS/STAT User's Guide. (2000). *Version 8*. Cary(NC): SAS Institute Inc.

Venkatraman, J. T., Toohey, T. & Clandinin, M. T. (1992). Does a threshold for the effect of dietary omega-3 fatty acids on the fatty acid composition of nuclear envelope phospholipids exist? *Lipids*, *27*(2), 94–97. doi:10.1007/bf02535806

Zhao, T., Ma, Y., Qu, Y., Luo, H., Liu, K., Zuo, Z. & Lu, X. (2016). Effect of dietary oil sources on fatty acid composition of ruminal digesta and populations of specific bacteria involved in hydrogenation of 18-carbon unsaturated fatty acid in finishing lambs. *Small Ruminant Research*, *144*, 126–134. doi:10.1016/j.smallrumres.2016.06.012

Варијације у саставу млијека коза услијед суплементације n-3 незасићеним масним киселинама

Весна Гантнер[1], Мирна Гавран[1], Маја Грегић[1], Божо Важић[3],
Ранко Гантнер[1], Клемен Поточник[2]

[1]*Свеучилиште Јосипа Јурја Штросмајера у Осијеку, Факултет агробиотехничких знаности, Хрватска*
[2]*Универзитет у Љубљани, Биотехнички факултет, Домжале, Словенија*
[3]*Универзитет у Бањој Луци, Пољопривредни факултет, Република Српска, БиХ*

Сажетак

Циљ овог истраживања био је утврдити учинак суплементације n-3 незасићених масних киселина (α-линолне, еикозапентаенојске и доксохексенска) на састав млијека (садржај млијечне масти и бјеланчевина) у млијечних коза. Надаље, анализирана је устрајност овог учинка након раздобља суплементације. Истраживање је проведено на млијечним козама (алпске и санске пасмине) узгајаним на фарми затвореног типа. Обзиром на раздобље покуса, узорковања млијека при мужњи (ујутро и навечер) проведена су у раздобљу прије суплементације (BS), тијеком суплементације (S) те након суплементације (AS). Надаље, обзиром на додатак суплемента, животиње су насумично распоређене у контролну скупину (G-4) без суплемента те три тестне скупине (G-1; G-2; G-3) гдје је суплемент који садржи PUFA додаван у раздобљу од пет дана. Добивени резултати индицирају да додатак PUFA у оброк резултира промјеном састава млијека коза. Додатак α-линолне киселине довео је до пораста садржаја и млијечне масти и бјеланчевина. Надаље, додатак докозахексаеноичне и еикосапентаенске киселине у оброк коза резултирао је смањењем садржаја млијечне масти, те повећањем садржаја бјеланчевина у млијеку. Утврђени трендови перзистирали су и након периода суплементације.

Кључне ријечи: млијечне козе, количина млијека, суплементација n-3 незасићених масних киселина