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The Effect of Fatty Acids in Goat Milk on Health

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1. Introduction

It has been recognized that components of foods can be contributing factors in human health and disease prevention. Based on the potential benefits to long-term human health there is interest in developing sustainable nutritional strategies for reducing saturated and increasing specific unsaturated fatty acids in ruminant milk. Despite the lower scale of milk production from goats compared with cows in Europe, there is an increasing interest in goat milk due to inherent species-specific biochemical properties that contribute to nutritional quality. Goat milk has been identified as a viable alternative for consumers that are sensitive or develop allergic reactions to bovine milk.

1.1. Synthesis and composition of goat milk fat

Fat composition in goat milk is one of the most important components of the technological, nutritional or dietetic quality of goat milk. Milk fat content in goat milk is high after parturition and then decreases during the major part of lactation. This is related to at least two phenomena: a dilution effect due to the increase in milk volume until the lactation peak, and a decrease in fat mobilization that decreases the availability of plasma non-esterified fatty acids, especially C18:0 and C18:1, for mammary lipid synthesis (Chilliard et al., 2003). Even that, total solids, fat, crude protein, lactose, and ash contents of goat milk are almost similar to cow milk, there are important differences in the individual fatty acids and casein fractions and fat globule sizes. Fat globules of goat milk are smaller in size and do not coalesce upon cooling because of lack of agglutinin, which is responsible for the aggregation of fat globules in cow milk.

Goat milk fat is composed primarily of triglycerids (or triacylglycerides) (in 98%) and in a small part from phospholipids and sterols. Triglycerids are synthesized on the outer surface of the smooth endoplasmic reticulum of the milk alveolar cells from precursor substances: fatty acids and glycerol. They are forming larger globules, which are travelling to the margin of cell. At the beginning, they attach to the membrane and they pass through. Then,

they are eliminated from the cell as fat globules of the milk. The synthesis is endogenous in a large extent, where the presence of the conjugated linoleic acid plays an important role (Hurley, 2009).

Fatty acids in goat milk are synthesized in epithelial cells of the mammary gland *de novo* or they are passing over from the blood (Chilliard et al., 2003). Two coenzymes have a major role in the synthesis of fatty acids in goat milk: acetyl-coenzyme A-carboxylase, which participates in the synthesis of fatty acids *de novo* and fatty acid synthase, which is a complex of enzymatic active substances and is responsible for the extension (elongation) of the fatty acid chain (Hurley, 2009). Fatty acids of exogenous origin are presented via the circulation to mammary epithelial cells either in the form of non-esterified fatty acids or esterified as the acyl groups of the triacylglycerol component of lipoprotein particles. In the mammary gland of ruminant animals, short and medium chain saturated fatty acids are the major products of *de novo* lipogenesis whereas plasma lipids contribute longer chain and mono unsaturated species. The acetate is the precursor of fatty acids synthesis in ruminants, while in monogastric animals, the precursor is glucose (Clegg et al., 2001).

1.2. Fatty acid composition in goat milk fat

Average goat milk fat differs in contents of its fatty acids significantly from average cow milk fat, being much higher in butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), linoleic (C18:2), but lower in stearic (C18:0), and oleic acid (C18:1) (Table 1). Three of the medium chain fatty acids (caproic, caprylic, and capric) have actually been named after goats, due to their predominance in goat milk. They contribute to 15% of the total fatty acid content in goat milk in comparison to 5% in cow milk (Haenlein, 1993). The presence of relatively high levels of medium chain fatty acids (C6:0 to C10:0) in goat milk fat could be responsible for its inferior flavour (Skjevdal, 1979).

Fatty acid	Goat milk ¹	Goat milk (from highland flock) ²	Goat milk (from mountain flock) ²	Cow milk ¹
C _{4:0} butyric	130	-	-	110
C _{6:0} caproic	90	-	-	60
C _{8:0} caprylic	100	106	85	40
C _{10:0} capric	260	433	321	80
C _{12:0} lauric	120	228	149	90
C _{14:0} myristic	320	441	392	340
C _{16:0} palmitic	910	984	990	880
C _{16:1} palmitoleic	80	-	-	80
C _{18:0} stearic	440	333	300	400
C _{18:1} oleic	980	-	-	840
C _{18:2} linoleic	110	103	76	80
C _{18:3} linolenic	40	32	26	50

Table 1. Fatty acid composition (mg FA 100 g⁻¹ milk) in goat milk fat in comparison to cow milk (1¹Posati & Orr, 1976; 2²Žan et al., 2005)

1.3. The effect of nutrition on goat milk fat and fatty acids composition

Nutrition (forage-to-concentrate ratio, type of forages, etc.) is the main environmental factor regulating milk fat synthesis and fatty acid composition in ruminants (Nudda et al., 2003; Bernard et al., 2009). Forage in the diet is known to affect milk fat composition responses to plant oils, including trans-18:1 and conjugated linoleic acid isomer concentrations. Inclusion of fat in the diet enhances milk fat secretion in the goat in the absence of systematic changes in milk yield and protein content (Bernard et al., 2009; Chilliard et al., 2003, 2007). Bernard et al. (2009) found out that, changes in goat milk fatty acid composition were dependent on forage type and plant oil composition, with evidence of an interaction between these nutritional factors. Responses to lipid supplements were characterised as a reduction in fatty acids synthesised de novo (C10:0–C16:0) and an increase in C18:0, cis-C18:1, conjugated linoleic acid and polyunsaturated fatty acid concentrations, indicating that plant oils can be used to effect potentially beneficial changes in milk fat composition without inducing detrimental effects on animal performance. Moreover, goats fed a high level of pasture forage had higher milk fat contents of C4:0, C6:0, C18:0, C18:1, C18:3, C20:0, iso-, ante-iso-, and odd fatty acids, but lower values of C10:0, C12:0, C14:0, C16:0, and C18:2, than those fed the low levels of forage. However, high levels of alfalfa forage also produced the lowest contents of the less desirable trans-C18:1 fatty acids (LeDoux et al., 2002). The conclusion was that decreasing the fibre content and increasing the grain part in the goat daily ration would lead to higher contents of the undesirable trans-C18:1 fatty acids in milk. The composition of goat milk fatty acids differed also in goats grazing one flock on highland (615-630 m altitude) and one flock on mountain (1060-1075 m altitude) pasture by Žan et al. (2005). The most abundant fatty acids in milk of both flocks were C16:0, C18:1, n-9, C14:0 and C10:0 (Table 1). The average content of saturated fatty acids was 74.52 and 73.05% in milk from the highland and mountain flocks, respectively. Three saturated fatty acids (caprylic (C8:0), capric (C10:0) and lauric acid (C12:0)), were present at significantly higher amounts in milk from the highland flock than in milk from the mountain flock. Monounsaturated fatty acids represented 20.49 and 22.32% and polyunsaturated fatty acids 3.73 and 3.24% of the milk from the highland and mountain flocks, respectively. Among the monounsaturated fatty acids, palmitoleic + palmitelaidic acid (C16:1, n-7) showed a significantly higher concentration in milk from mountain flock than in milk from the highland flock. The content of linolelaidic acid (C18:2, n-6) was significantly higher in comparison to milk from the highland flock. The average quantity (32 mg 100 g⁻¹ milk) of essential α -linolenic acid (C18:3, n-3) was slightly higher in milk of the highland flock than in milk from the mountain flock (26 mg 100 g⁻¹ milk). Hou et al. (2011) stated that the supplementation of fish oil can significantly increase the production of cis-9, trans-11 conjugated linoleic acid, and trans-11 C18:1, while lowering the amount of trans-10 C18:1 and trans-10, cis-12 conjugated linoleic acid in the ruminal fluid of goats. Increased cis-9, trans-11 conjugated linoleic acid, and trans-11 C18:1 can lead to a higher output of cis-9, trans-11 conjugated linoleic acid in milk product, and the decrease in trans-10 C18:1 and trans-10, cis-12 conjugated linoleic acid supports the role of fish oil in the alleviation of milk fat depression.

1.4. Conjugated linoleic acid

Conjugated linoleic acid consists of a series of positional and geometric dienoic isomers of linoleic acid that occurs naturally in foods. It is a product of biohydrogenation in the rumen of ruminants and has a great influence on synthesis of fatty acids in milk in low concentrations (Bessa et al. 2000; Chouinard et al. 1999; Griinari & Bauman, 1999; Griinari et al. 2000; Khanal & Dhiman, 2004). Actually, the conjugated linoleic acid found in goat milk fat originate from two sources (Griinari & Bauman, 1999). One source is conjugated linoleic acid formed during ruminal biohydrogenation of linoleic acid (C18:2 n-6) that leads first to vaccenic (trans-11 C18:1) and finally to stearic acid (C18:0) (Nudda et al., 2003). The second source is conjugated linoleic acid synthesized by the animal's tissues from trans-11 C18:1, another intermediate in the rumen biohydrogenation of unsaturated FA. Thus, the uniqueness of conjugated linoleic acid in food products derived from ruminants relates to the incomplete biohydrogenation of dietary unsaturated fatty acids in the rumen. Ruminal biohydrogenation combined with mammary lipogenic and Δ -9 desaturation pathways considerably modifies the profile of dietary fatty acids and thus milk composition (Chilliard et al., 2007).

Dietary sources from ruminants such as milk, cheese and meats contain more conjugated linoleic acid than foods of non-ruminant origin (Bessa et al. 2000; Khanal & Dhiman, 2004). The increase of linoleic acid intake is one of the feeding strategies for conjugated linoleic acid enrichment in ruminant fat since linoleic acid is the main precursor of conjugated linoleic acid (Bessa et al., 2000). The main available sources of linoleic acid in animal feeds are cereal and oilseed grains or oils obtained from these. Goat milk conjugated linoleic acid content increases sharply after either vegetable oil supplementation (Bernard et al., 2009) or fresh grass feeding containing unsaturated fatty acids, but does not change markedly when goats receive whole untreated oilseeds (Chilliard et al., 2003). Mir et al. (1999) found that it is possible to increase conjugated linoleic acid content of goat milk by manipulation of dietary regimen such as supplementation with canola oil. The pasture has major effects by decreasing saturated fatty acids and increasing fatty acids considered as favourable for human health (C9-18:1, C18:3n-3 and C9t11-CLA), compared to winter diets, especially those based on maize silage and concentrates (Chilliard et al., 2007). Investigations have shown that milk fat conjugated linoleic acid content can be also enhanced by manipulation of the rumen fermentation (Bessa et al., 2000; Griinari et al., 1999) or by direct addition of a dietary supplement of conjugated linoleic acid (Lock et al., 2008).

1.5. Effect of fatty acids on health

Milk, apart from its nutritional traits, contains substances which have beneficial effects on human health and is, therefore, considered essential to a correct nutrition. In particular, in milk are present vitamin A, vitamin E, β -carotene, sphingomyelins, butyric acid, and conjugated linoleic acid, all with a strong antitumor effect (Parodi, 1999). Different FA (short and medium chain, saturated, branched, mono and polyunsaturated, *cis* and *trans*, conjugated) in the lipid fraction of milk are potentially involved as positive or negative

predisposing factors for human health (Parodi, 1999; Williams, 2000). In this respect, conjugated linoleic acid is the most characteristic one. One of the goat milk significance in human nutrition is treating people afflicted with cow milk allergies and gastro-intestinal disorders, which is a significant segment in many populations of developed countries. Fat in goat milk is more digestible than bovine milk fat which may be related to the lower mean milk fat globule size, higher C8:0–C10:0 concentrations and a larger proportion of short- and medium-chain fatty acids (Chilliard et al., 2006 as cited in Bernard et al., 2009). Because of predominance of smaller fat globules in goat milk, it is easier to digest than cow milk and this may be attributed to faster lipase activity on smaller fat globules due to a greater surface area (Chandan et al., 1992). Goat milk is therefore recommended for infants, old, and convalescent people.

The physiological and biochemical facts of the unique qualities of goat milk are just barely known and little exploited, especially not the high levels in goat milk of short and medium chain fatty acids, which have recognized medical values for many disorders and diseases of people (Haenlein, 2004). Goat milk exceeds cow and sheep milk in monounsaturated, polyunsaturated fatty acids, and medium chain triglycerides, which all are known to be beneficial for human health, especially for cardiovascular conditions. Capric, caprylic acids and medium chain triglycerides have become established medical treatments for an array of clinical disorders, including malabsorption syndromes, chyluria, steatorrhea, hyperlipoproteinemia, intestinal resection, premature infant feeding, non-thriftiness of children, infant malnutrition, epilepsy, cystic fibrosis, coronary by-pass, and gallstones, because of their unique metabolic ability to provide direct energy instead of being deposited in adipose tissues, and because of their actions of lowering serum cholesterol, inhibiting and limiting cholesterol deposition (Alferrez et al., 2001; Greenberger & Skillman, 1969; Kalser, 1971; Schwabe et al., 1964; Tantibhedhyanangkul & Hashim, 1978).

Conjugated linoleic acid was recognized as having antioxidative and anticarcinogenic properties in animal model studies (Ip et al., 1991; Jiang et al., 1996; Parodi, 1997). Several *in vitro* and *in vivo* studies showed also antiatherogenic, anti-obesity, anti-diabetes and immune-stimulating properties of conjugated linoleic acid (McGuire & McGuire, 1999). By Parodi (1997), conjugated linoleic acid inhibited proliferation of human malignant melanoma, colorectal, breast and lung cancer cell lines. Anticarcinogenic effects of conjugated linoleic acid appear to be dose dependent, from 0.1 to 1% in the diet (Ip et al., 1991). Conjugated linoleic acid reduced the incidence of chemically induced mouse epidermal tumors, mouse forestomach neoplasia and aberrant crypt foci in the rat colon. They have been also shown to stimulate immune response and protect against arteriosclerosis (Cook et al., 1993; Lee et al., 1994). When rabbits were fed conjugated linoleic acid, LDL cholesterol to HDL cholesterol ratio and total cholesterol to HDL cholesterol ratio were significantly reduced. Examination of the aortas of conjugated linoleic acid fed rabbits showed less atherosclerosis (Lee et al., 1994).

Somatic cells in milk are the total sum of white blood cells present in milk and udder epithelial cells, which may be an indicator of the udder health status (Das & Singh, 2000; Manlongat et al., 1998; Zeng & Escobar, 1996; Wilson et al., 1995). They are present in milk

all the time. In cows, a somatic cell count above the regulatory standard is generally considered as an indication of mastitis. An increased number of somatic cell count is either the consequence of an inflammatory process due to the presence of an intramammary infection or under non-pathological conditions due to physiological processes such as oestrus or advanced stage of lactation. For this reason, the somatic cell count of milk represents a sensitive marker of the health of the udder and is considered a useful parameter to evaluate the relationship between intramammary infection and changes in milk characteristics. The standard for the permissible number of somatic cell count for cow milk exists, while it is still under study for goat milk due to considerable fluctuations. When the udder is tired during late lactation, the number of somatic cells in normal conditions can considerably enlarge, and approximately 80% of the cells may be polymorphonuclear leukocytes (Manlongat et al., 1998). The same authors found that normal nonmastitic late-lactation-stage goat milk is significantly higher in polymorphonuclear leukocytes chemotactic activity than early-lactation-stage goat milk. The chemotactic factor(s) present in the milk of normal late-lactation-stage goats is nonpathological and may play a physiologic regulatory role in mammary gland involution. On the other hand, the increase of leucocytes is a response to the inflammatory process in the mammary gland or somewhere in the body. The number of leucocytes increases due to bacterial infections, but it could also be increased due to the stage of lactation, age of the animal, stress, season of the year, nutrition and udder injuries. The variability of somatic cell count in goat milk is very high, which exists among the animals and within the time span of individual animals (Das & Singh, 2000). Therefore, it is important to determine how nutrition can influence the reduction of somatic cell count in goat milk. Gantner & Kompan (2009) found that a five-day supplementation of α -linoleic acid in Alpine goat diet had a significant effect on lower somatic cell count in milk. Based on this experiment, it was concluded that α -linoleic acid supplementation had no effect on milk yield; it had low effect on milk components and significant effect on somatic cell count. A decrease in somatic cell count was determined in the 1st day of the treatment period and continued until 30th day after the treatment period. The supplementation of the goat diet with α -linoleic acid could be used as a method of choice for reduction of somatic cell count in goat milk.

The aim of our study was therefore to ascertain the changes in goat milk yield and its contents of fat, protein, lactose, dry matter, somatic cell count, and total number of microorganisms when goats are supplemented with the following fatty acids: α -linoleic acid, eicosapentanoic acid, and docosahexanoic acid and how these three fatty acids influence on the content of particular fatty acids during and after the supplementation.

2. Material and methods

2.1. Material

The research was performed on the farm with 90 Slovenian Alpine and Slovenian Saanen goats. Goats were machine milked. During the experiment, goats were in different stages of lactation. The average body weight of the goats was 51 ± 6 kg. All kids were weaned. Goats

were arranged into three pens according to their stage of lactation, namely, after kidding from the forth to the tenth week of lactation (pen A), from the 11th to the 20th week of lactation (pen B), and after the 20th week of lactation (pen C). Goats were milked twice a day, at 6 a.m. (± 30 min) and at 6 p.m. (± 30 min). Diet was composed from hay (2 kg/animal/day) which was given to goats twice a day. Goats were supplemented with feed mixture at milking parlor during the milking time. Supplemental feed mixture contained 50% of grounded maize grains, 30% of dried beet pulp, and 20% of wheat bran. Goats from pen A were supplemented with 500 g, goats from pen B with 350 g, and goats from pen C with 250 g of feed mixture. Vitamin-mineral supplement and water were offered to goats *ad libitum*. After the tenth day preparing period, 62 goats from pens A and B were selected and randomly arranged into four experimental groups. At the beginning of the experiment (September 17th, 2000), goats were 28 to 105 days after kidding. The experiment lasted 63 days. During this time, experimental goats were added fats or oils.

2.2. Methods

2.2.1. Measuring performance and milk sampling

The whole experiment was performed in three periods:

1st period: Preparatory period - measuring before adding fats or oils. The preparatory period lasted 10 days. During this period, milk yield in goats was measured, milk samples were collected, and animals were adapting to the working group. Goats were adapted to the work and people after a week, so they were not under the stress any more. Milk yield was measured every day at morning and evening milking, when 70 ml of milk sample was taken for the analysis of milk content, somatic cells, and bacteriological analysis, and 2 ml for fatty acid content analysis.

2nd period: Experimental period – adding fatty acids. After the tenth day preparing period, 62 goats from pens A and B were randomly selected into four experimental groups, named EPA, ALFA, DHA, and KONT. There were 15 goats in groups EPA, ALFA, and DHA and 17 goats in the group KONT. Supplementation of the fats was performed 5 days (from the 11th to the 15th day), after morning milking in groups EPA, ALFA, and DHA. Each goat was cached and individually administered the appropriate quantity of fatty acids into its mouth with a special sound. Group EPA was receiving a preparation rich in eicosapentaenoic acid (EPA; 20 g/day), group ALFA was receiving a linseed oil rich in α -linoleic acid (ALA; 20 g/day), and group DHA was receiving a preparation rich in docosahexaenoic acid (DHA; 20 g/day). Group KONT was a control group, which was receiving no preparation. Measuring of the milk yield and collecting milk samples was followed the same procedure as in the first period.

3rd period: This period lasted from the 16th day, after the end of administering fatty acids to goats. Milk yield measuring and milk samples collection was continuing until the 20th day. From the 21st day of the experiment, milk yield measuring and milk samples collection was performing every five days, at the morning and evening milking, until the end of the

experiment (63rd day). All together, 30 morning and 30 evening records were collected by each goat.

2.2.2. Milk yield measuring

There were 90 goats all together in the flock, which were milked on the milking parlor with 24 places for milking goats connected to milk pipeline. Goats were milked every morning between 5:40 and 7:20 a.m. and every evening between 6:20 and 8:00 p.m. A measuring gauged flask was connected to milking unit to measure milk yield. Milk yield was written down for every goat. A milk sample was also taken for the analysis. During the experiment, 30 daily records were collected for every goat, which means 60 records for each goat and 60 milk samples by 70 ml for milk analysis (sample A) and 60 samples by 2 ml (sample B) for fatty acid analysis. The preservative azidiol on the basis of NaN_3 in concentration 0.02% with the addition of chloramphenicol for the stabilization of microorganisms was added to the sample A. For every 50 to 70 ml of the milk sample, 0.2 ml of the preservative was added. Milk samples A were then delivered to the Laboratory for dairying, while milk samples B were delivered to the Chemical laboratory at Biotechnical Faculty in Ljubljana.

2.2.3. Analyses of milk samples

Chemical composition, somatic cell count, and total number of microorganisms: Fat, protein, lactose, and dry matter content, somatic cell count and total number of microorganisms were determined in the collected milk samples A in the Laboratory of dairying at Biotechnical Faculty in Ljubljana. Furthermore, fatty acid composition of milk lipids was determined. Chemical composition of goat milk was determined by the instrument MilkoScan 133 B, which operates on the principle of infrared spectrometry. Somatic cell count was determined using apparatus Fossomatic 5000, which operates on the basis of automatic epifluorescent technique, by the principle of flow cytometry. The total number of microorganisms was determined using the apparatus Bactoscan 8000, type 27000.

Fatty acid composition of milk lipids: Milk samples B were stored in liquid nitrogen immediately after milk recording. They were stored then in freezer chamber at -70°C until the analysis. Before the analysis, milk samples were warmed to $38-40^\circ\text{C}$ in water bath and mixed up. After that, 500 mg of the milk sample were weighed out into tubes, where $300\ \mu\text{l}$ of methylenchloride and 3 ml of fresh prepared 0.5M of sodium hydroxide in methanol were added. To determine the fatty acid composition of milk lipids, the analysis of methyl esters of fatty acids was done. This analysis was performed on gas chromatograph Hewlett Packard HP AGILENT 6890 SERIES GC SYSTEM, USA. Processing of chromatographic data was conducted using ChemStation Plus software. Furthermore, factor of the responsiveness of the flame ionization detector was determined. Total lipids in the sample are composed of both fatty acids and glycerol from triglycerids, phosphate from phospholipids, and sterol. For the calculation of the fatty acid value in the sample in mg, special factors are used, which express the proportion of acids in total fat.

2.2.4. Statistical analysis of the data

The statistical package SAS (SAS/STAT, 2000) and partly the statistical package S-PLUS (1966) were used to analyse the data. The statistical analysis did not include records collected during the first six days of the preparation period. In the meantime, the situation in the stable was stabilizing and the team who participated in the experiment was introducing in the everyday milk measuring and collecting samples.

Due to the large fluctuations in individual values of the somatic cell count and number of microorganisms among animals and among observations within animals, we analyzed each animal individually as its time series, and for the most variable ones the logarithm of the values was found ($X = \log_{10}Y$).

The time series were first standardized (S) in the way that last four days (from the 7th to 10th day) of the preparatory period (before supplementing with fatty acids) were taken as a starting point. Mean value of this period was calculated by the median (Me), the measure of variability was the average absolute deviation (AD). In this way we reduced the impact of outliers. Although, it is usual to standardize by the average and standard deviation, we decided for median and absolute deviation. In this way, the standardized time series for the animal was calculated using the following equation:

$$S = ((X - \text{Me}) / \text{AD}) \dots \quad (1)$$

In this way, the standardized time series (S) are comparable for animals with different values. Then, we calculated the median for the three periods on the standardized time series:

- median for the period from the seventh to tenth day of the experiment (preparatory period), which was in all cases zero (=0);
- median for the period from 11th to the 15th day of the experiment (the period of supplementing with fatty acids);
- median for the period from the 16th to the 63rd day of the experiment (the post supplementation period of the fatty acids).

For each animal, the corresponding median has become an input data for the statistical analysis. In this way, we analyzed milk yield (ml), the content of milk proteins (g/100 ml), milk fat (g/100 ml), milk lactose (g/100 ml), dry matter (g/100 ml), non-fat dry matter (g/100 ml), total number of microorganisms ($n \cdot 10^3/\text{ml}$), and somatic cell count ($n \cdot 10^3/\text{ml}$) in milk.

In this way, a comparison of groups with a simple analysis of variance was made where the zero assumption was checked for that the averages by groups were the same. If a statistically significant difference test was found (5% level of significance was considered), then the groups were compared also by the Duncan test or by the contrast analysis, where each group was compared with the control group.

All other traits were analyzed by the GLM procedure (General Linear Model) with statistical package SAS, which included the impacts of the group (4) and period (3). Differences

among groups were estimated by the linear contrasts, while connections between the properties were calculated by the Pearson correlation coefficient. The limit of statistical significance was taken at $P < 0.05$ and highly statistically significance was taken at $P < 0.001$.

3. Results and discussion

3.1. Milk yield and the chemical composition of milk

The average milk yield and its content of fat, proteins, lactose, dry matter, non-fat dry matter, total number of microorganisms, and somatic cell count in different periods of the experiment by groups is shown in Table 2. In the preparatory period, only somatic cell count statistically significantly differed among groups. Statistically significant differences among groups in the experimental period appeared in dry matter, somatic cell count, and logarithm of the somatic cell count. In the third period of the experiment, statistically significant differences among groups appeared in the majority of observed traits.

It seems that the short time fatty acid supplementation into goat's diet does not negatively affect their milk yield. Milk yield did not vary statistically significant during the observed period (Table2). As found by Sampelayo et al. (2002), the supplemented fatty acids into the diet of Granadina goats did not affect their milk yield and the content of fat, proteins, lactose, and dry matter in milk.

Group	EPA	ALFA	DHA	KONT	EPA	ALFA	DHA	KONT	EPA	ALFA	DHA	KONT
Trait/Period	1	1	1	1	2	2	2	2	3	3	3	3
Milk (ml)	780 ^a	748 ^a	869 ^a	766 ^a	790 ^a	708 ^a	888 ^a	824 ^a	765 ^a	719 ^a	884 ^a	789 ^a
Fat (g/100 ml)	3.05 ^a	3.15 ^a	3.00 ^a	2.99 ^a	2.65 ^a	3.40 ^b	2.52 ^a	2.91 ^a	2.84 ^a	3.30 ^b	2.77 ^a	3.06 ^a
Proteins (g/100 ml)	2.93 ^a	3.12 ^a	2.98 ^a	3.01 ^a	3.01 ^a	3.21 ^b	3.01 ^a	3.06 ^a	3.15 ^a	3.28 ^a	3.29 ^a	3.07 ^b
Lactose (g/100 ml)	4.59 ^a	4.55 ^a	4.49 ^a	4.53 ^a	4.58 ^a	4.54 ^a	4.48 ^a	4.44 ^a	4.50 ^a	4.54 ^a	4.44 ^a	4.46 ^a
NFDM (g/100 ml)	8.32 ^a	8.48 ^a	8.26 ^a	8.33 ^a	8.39 ^a	8.55 ^a	8.29 ^a	8.30 ^a	8.45 ^a	8.62 ^b	8.53 ^a	8.33 ^a
DM (g/100 ml)	11.37 ^a	11.62 ^a	11.26 ^a	11.33 ^a	11.04 ^a	11.76 ^b	10.81 ^a	11.21 ^a	11.29 ^a	11.92 ^b	11.30 ^a	11.39 ^a
MO (n*10 ³ /ml)	653 ^a	609 ^a	498 ^a	505 ^a	315 ^a	334 ^a	350 ^a	494 ^a	266 ^a	267 ^a	267 ^a	347 ^a
SCC (n*10 ³ /ml)	1316 ^a	1095 ^a	585 ^b	526 ^b	1631 ^a	975 ^b	1166 ^a	1258 ^a	1531 ^a	915 ^b	1884 ^a	1364 ^a
log ₁₀ _MO (n*10 ³ /ml)	2.64 ^a	2.62 ^a	2.47 ^a	2.58 ^a	2.44 ^a	2.44 ^a	2.48 ^a	2.62 ^b	2.35 ^a	2.34 ^a	2.34 ^a	2.48 ^a
log ₁₀ _SCC (n*10 ³ /ml)	2.69 ^a	2.68 ^a	2.62 ^a	2.54 ^a	2.82 ^a	2.72 ^a	2.80 ^a	2.99 ^a	2.83 ^a	2.77 ^b	2.88 ^a	2.99 ^a

^{a,b} – values which are not marked with the same letter are statistically significantly different at least $P < 0.05$

NFDM – non-fat dry matter; DM – dry matter; SCC – somatic cell count; MO – microorganisms;

Table 2. Average values of the observed traits in different periods of the experiment by groups

Milk fat yield statistically significantly increased in ALFA group from 3.15 to 3.40 g/100 ml on average when goats were supplemented with linseed oil rich in α -linoleic acid (Table 2) and it slightly decreased to 3.30 g/100 ml until the third period of the experiment. In groups EPA and DHA, milk fat yield firstly decreased, while it increased slightly after the end of supplementation with fatty acids.

There were no statistical significant differences among the groups of goats in milk protein yield before the supplementation with fatty acids (Table 2). During the supplementation of goats with fatty acids, milk protein yield increased and it was increasing also after the end of supplementation. Group ALFA had the highest protein yield in milk in the whole time of the experiment.

In general, lactose in milk varies little, what was confirmed also in our research. There were no statistical significant differences in lactose yield among the observed groups, neither during the supplementation with fatty acids nor after that (Table 2).

Non-fat dry matter increased during the experiment in all observed groups which were supplemented with fatty acids, but not in the control group KONT (Table 2). Differences among groups were not statistically significant. Total dry matter decreased after supplementing with fatty acids in groups EPA, DHA, and KONT, while it increased in ALFA group. After the end of supplementing with fatty acids, total dry matter increased in all groups. Group ALFA statistically significantly differed in milk dry matter from other observed groups in the second and third period of the experiment.

The number of microorganisms in milk mostly depends on milking hygiene, which includes staff, animals, facilities, equipment, hygiene maintenance, and cleaning of the equipment. It also depends on the health of the udder and the presence of mastitis. Soon after the beginning of the experiment, the hygiene and cleaning improved, and the number of microorganisms in milk decreased (Table 2). There was no mastitis detected in the whole time of the experiment. No statistically significant differences were noticed among groups in the number of microorganisms in milk.

Somatic cell count was one of the most variable traits in our experiment, since we found that values ranged from 13.000 to 24,312.000 of somatic cells in ml of milk. Despite the great variability, transformation of somatic cell count to the logarithmic value enabled to find the possible impacts of supplementation with fatty acids on somatic cell count (Figure 1). Preliminary report by Košmelj et al. (2001) showed the impact of supplementing alpha-linolenic fatty acid to goats, which was reflected in a reduction of the number of somatic cells during the supplementation and four weeks after.

The average values for medians during the supplementation with fatty acids (Me_1) and for medians five days after the supplementation with fatty acids (Me_2) are shown in Table 3. Results showed statistical significant differences among groups of goats for medians during the supplementation with fatty acids and also for medians five days after the supplementation with fatty acids. The average of medians (Me_1 and Me_2) in group ALFA is negative, so it could be affirmed, that the supplementation of linseed oil rich in α -linoleic acid decreases the number of somatic cell count in milk.

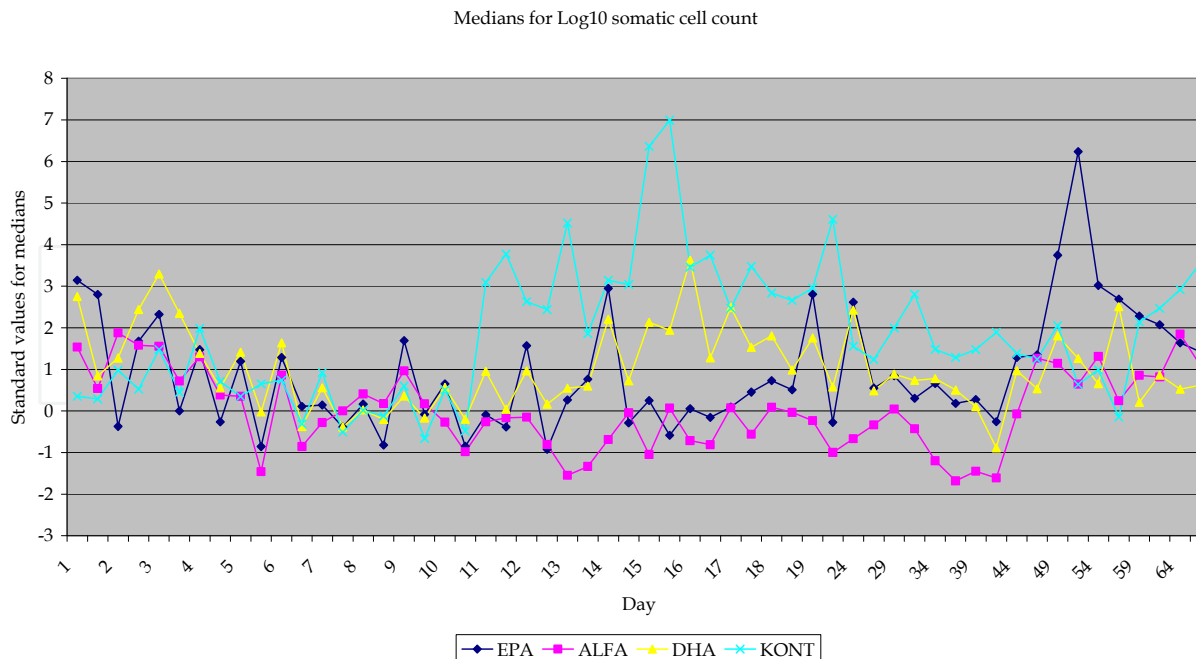


Figure 1. Standardization and log₁₀ value median for number of somatic cells by groups

Period / Group	EPA	ALFA	DHA	KONT
Average for Me ₁	1.01 ^b	-3.11 ^a	1.68 ^b	3.52 ^b
Average for Me ₂	1.75 ^b	-2.47 ^a	1.78 ^b	3.20 ^b

^{a, b} - Groups with different letter are statistically significantly different ($P < 0.05$)

Table 3. Average value of Me₁ in Me₂ in different group

On average, somatic cells in goat milk are present in a greater number than in cow milk. Zeng et al. (1997) reported that 17% of goat milk samples recorded on goat farms which are members of the Association of goat farmers in the U.S. exceeded the standard 1.0×10^6 of somatic cells ml^{-1} when the experiment of daily monitoring of somatic cells in milk was carried out.

Das & Singh (2000) studied somatic cells in goat milk and electrical conductivity of milk. In the blood samples total leucocytes and differential leucocytes (lymphocytes, monocytes, neutrophils, eosinophil, and basophils) were also determined. Somatic cell count in goat milk was high during early lactation and decreased subsequently as the lactation advanced. There were found individual variations ($P < 0.01$) in somatic cell counts between different lactation periods as well as among and within animals. For example, one goat had very high somatic cell count in comparison to other goats from the beginning to the end of the experiment. The goat was then tested for mastitis using California mastitis test and it was found to have normal milk. Similar results were found in our experiment. Total leucocyte count in blood also decreased as the lactation progressed and remained fluctuated during late lactation in the study by Das & Singh (2000). Lymphocytes and neutrophils were low during early lactation and with establishment of lactation stabilized to normal levels. Protein content of milk did not vary during different periods of lactation. However, lactose

decreased and fat percent increased with advanced lactation. It is interesting that the connection between somatic cell count and milk yield and between somatic cell count and milk composition was not found in any stage of lactation.

Mastitis is typically associated with a large number of somatic cells in small ruminants. In our experiment, the number of somatic cells significantly reduced only in the ALFA group and lasted statistically significant 39 days after the supplementation with fatty acids. For α -linolenic fatty acid is known, that it could incorporate into phospholipids five hours after ingestion (Adam et al., 1986). The other two, eicosapentaenoic acid and docosahexaenoic acid can incorporate into phospholipids only after a few days supplementation. The statistically significant effect of the α -linolenic fatty acid only on somatic cell count could be explained by the rapidness of incorporation into membrane phospholipids of this fatty acid.

The fluctuations of the somatic cell count in goat milk are subjected to many influences. Researchers have not explored other reasons for the number of somatic cells in goat milk except the hygiene measures. Ruminants are in the last 20 years fed adding n-3 fatty acids to improve the fatty acid composition of milk and meat, but the impact on the number of somatic cells have not been monitored. Our experiment clearly shows that the supplementation of the α -linolenic fatty acid had a relatively long time impact on reducing somatic cell count or to a low level of somatic cells in milk. The interpretation may be possible, that we achieved a more appropriate relationship between n-3 and n-6 long chain fatty acids with the supplementation of α -linolenic fatty acid which was not provided by the diet.

3.2. Composition of fatty acids in goat milk

Chemical analysis of goat milk fat was done for fatty acids from 10:00 to 24:6, n-9. The fat composition of goat milk was studied by each milking during the experiment time. Therefore, values listed below (Table 3) represent the percentage of the all analyzed fatty acids rather than total fat in goat milk.

During our experiment, there was from 9.0 to 14.0 wt % of the **capric** acid (10:0) in the goat milk fat. Some authors (Hurley, 2009; Jandal, 1996; Sanz Sampelayo et al., 2002) indicated values from 8.4 to 11.1%. EPA group had the lowest level of capric acid before supplementing with fatty acids, while its level exceeded groups ALFA and KONT during the supplementation and declined to the lowest level among groups in the last period of the experiment. DHA group had the highest level of the capric acid during the supplementation with fatty acids as well as all the time after the supplementation. It is known that goat milk has more short-chain fatty acids (C_{4:0} to C_{10:0}) than cow's milk, which are easier to digest than long-chain fatty acids.

We found that the **lauric** acid (12:0) in goat milk fat presented between 3.8 and 7.7 wt %. During the supplementation with fatty acids, the lauric acid increased for 2% in DHA group and for 1% in EPA group. The increase in EPA group lasted two days after the end of the supplementation, and four days in DHA group. Hurley (2009) found that there is 3.3% of the lauric acid in goat milk fat, Jandal (1996) reported about 6.0%, while Sanz Sampelayo et al. (2002) found from 4.69 to 5.11% of the lauric acid in goat milk fat.

Experimental period	1	1	1	1	2	2	2	2	3	3	3	3
FA / GROUP	EPA	ALFA	DHA	KONT	EPA	ALFA	DHA	KONT	EPA	ALFA	DHA	KONT
10:0	9.55 ^a	11.63 ^a	10.84 ^a	11.16 ^a	11.64 ^a	11.20 ^a	13.13 ^b	11.60 ^a	9.84 ^a	10.64 ^a	12.09 ^b	10.08 ^a
12:0	4.43 ^a	6.06 ^a	4.97 ^a	5.38 ^a	5.41 ^a	5.65 ^a	6.56 ^a	6.06 ^a	4.93 ^a	5.56 ^a	6.21 ^a	4.96 ^a
14:0	10.79 ^a	12.31 ^a	11.44 ^a	11.88 ^a	11.88 ^a	10.90 ^b	11.62 ^a	12.49 ^a	11.25 ^a	11.25 ^a	11.39 ^a	11.14 ^a
16:0	25.62 ^a	24.92 ^a	27.61 ^a	26.01 ^a	23.98 ^a	22.79 ^a	25.24 ^a	25.04 ^a	24.75 ^a	23.29 ^a	24.12 ^a	23.81 ^a
16:1, n-7	1.25 ^a	1.19 ^a	1.32 ^a	1.18 ^a	1.24 ^a	1.18 ^a	1.51 ^b	1.16 ^a	1.22 ^a	1.25 ^a	1.57 ^b	1.25 ^a
18:0	11.09 ^a	9.98 ^a	10.65 ^a	10.24 ^a	7.26 ^b	10.32 ^a	4.23 ^b	9.54 ^a	11.14 ^a	11.08 ^a	7.31 ^b	11.04 ^a
18:1, n-9c, 18:1, n-9t, 18:1, n-12t, 18:1, n-7c	26.19 ^a	23.08 ^a	22.29 ^a	23.68 ^a	21.89 ^a	22.80 ^a	19.94 ^b	22.62 ^a	24.25 ^a	24.58 ^a	22.50 ^a	26.00 ^a
CLA (1)	0.82 ^a	0.81 ^a	0.74 ^a	0.79 ^a	1.73 ^b	1.35 ^b	2.89 ^b	0.92 ^a	1.19 ^b	1.06 ^a	2.50 ^b	0.93 ^a
18:2, n-6c	2.31 ^a	2.14 ^a	2.22 ^a	2.17 ^a	2.81 ^b	3.10 ^b	2.40 ^a	2.19 ^a	2.22 ^a	2.48 ^a	2.39 ^a	2.55 ^a
18:3, n-3	0.70 ^a	0.57 ^a	0.53 ^a	0.52 ^a	0.97 ^a	2.98 ^b	0.60 ^a	0.80 ^a	0.87 ^a	0.95 ^b	0.66 ^a	0.78 ^a
18:3, n-6	0.05 ^a	0.04 ^a	0.03 ^a	0.00 ^a	0.14 ^a	0.29 ^b	0.14 ^a	0.17 ^a	0.08 ^a	0.12 ^a	0.09 ^a	0.04 ^a
20:3, n-3	0.03 ^a	0.02 ^a	0.02 ^a	0.02 ^a	0.32 ^b	0.03 ^a	0.04 ^a	0.03 ^a	0.12 ^b	0.04 ^a	0.02 ^a	0.03 ^a
20:3, n-6	0.02 ^a	0.03 ^a	0.02 ^a	0.02 ^a	0.06 ^b	0.03 ^a	0.04 ^a	0.02 ^a	0.03 ^a	0.02 ^a	0.04 ^a	0.03 ^a
20:4, n-6	0.25 ^a	0.21 ^a	0.22 ^a	0.21 ^a	0.39 ^b	0.21 ^a	0.47 ^b	0.20 ^a	0.32 ^b	0.22 ^a	0.30 ^b	0.23 ^a
20:5, n-3	0.08 ^a	0.07 ^a	0.07 ^a	0.06 ^a	2.41 ^b	0.14 ^a	0.48 ^a	0.13 ^a	0.50 ^b	0.15 ^a	0.30 ^a	0.09 ^a
22:3, n3	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.04 ^b	0.00 ^a	0.07 ^b	0.00 ^a	0.00 ^a	0.00 ^a	0.07 ^b	0.02 ^a
22:4, n6	0.07 ^a	0.08 ^a	0.07 ^a	0.07 ^a	0.10 ^a	0.07 ^a	0.10 ^a	0.09 ^a	0.10 ^a	0.09 ^a	0.10 ^a	0.10 ^a
22:5, n-3	0.18 ^a	0.15 ^a	0.14 ^a	0.13 ^a	0.64 ^b	0.19 ^a	0.49 ^b	0.15 ^a	0.60 ^b	0.24 ^a	0.33 ^b	0.18 ^a
22:6, n-3	0.07 ^a	0.06 ^a	0.05 ^a	0.06 ^a	0.13 ^a	0.16 ^a	2.27 ^b	0.13 ^a	0.15 ^a	0.11 ^a	0.79 ^b	0.10 ^a
n-3	0.99 a	0.81 a	0.76 a	0.73 a	4.13 b	3.34 b	1.68 a	1.11 a	1.99 b	1.38 a	1.38 a	1.10 a
n-6	2.70 a	2.50 a	2.56 a	2.47 a	3.47 b	3.70 b	3.17 b	2.67 a	2.69 b	2.93 a	2.92 a	2.95 a
n-3/n-6	0.37 a	0.32 a	0.30 a	0.30 a	1.19 a	0.90 b	0.53 b	0.42 a	0.74 b	0.47 a	0.47 a	0.37 a
n-3 : n-6 (1:X)	2.73 a	3.09 a	3.37 a	3.38 a	0.84 b	1.11 b	1.89 a	2.41 a	1.35 b	2.12 a	2.12 a	2.68 a
LC PUFA n-3	0.36 a	0.30 a	0.28 a	0.27 a	3.29 b	0.52 a	3.35 b	0.44 a	1.27 b	0.54 a	1.51 b	0.42 a
LC PUFA n-6	0.34 a	0.32 a	0.31 a	0.30 a	0.52 b	0.31 a	0.63 b	0.31 a	0.39 a	0.33 a	0.44 a	0.36 a
LC PUFA n-3/ LC PUFA n-6	1.06 a	0.94 a	0.90 a	0.90 a	6.33 b	1.68 a	5.32 b	1.42 a	3.26 b	1.64 a	3.43 b	1.17 a
LC n-3 : n-6 (1:X)	3.11 a	2.93 a	2.91 a	3.00 a	12.17 b	5.41 a	8.44 b	4.58 a	8.35 b	4.96 a	7.80 b	3.24 a

^{a b} – values which are not marked with the same letter, are statistically different at least $P < 0.05$

FA – fatty acid; CLA – conjugated linoleic acid; LC PUFA – long chain polyunsaturated fatty acid

Table 4. Average values of fatty acids, secreted in milk in different periods of the experiment by groups (wt %)

Myristic acid (14:0) in goat milk fat represented from 10.0 to 13.5 wt % of fatty acids. The content was similar than in Sanz Sampelayo's et al. (2002) research. Throughout supplementing the fatty acids, a statistically significant reduction of myristic acid level in milk fat was noticed only in the ALFA group ($p < 0.05$). Other variations were not statistically significant and the level of myristic acid was similar among groups. Myristic content in goat milk fat was very stable during the experiment.

Miristoleic acid (14:1) in goat milk fat was detected in the content from 0.12 to 0.40 wt %, while Sanz Sampelayo et al. (2002) listed the values between 0.41 and 0.64%. We have not observed differences among groups and even daily fluctuations of miristoleic acid in goat

milk fat were very small. Miristoleic acid values were fluctuating at least in DHA group. Differences among groups were not found in any period of the experiment.

There was between 20 and 29 wt % of the **palmitic** acid (16:0) in the goat milk fat. Sanz Sampelayo et al. (2002) indicated values of the palmitic acid between 24.6 and 27.7%. There were no statistically significant differences observed among groups before the supplementation of the fatty acids to the goat diet. There was a trend of decreasing values during and immediately after the supplementation of fatty acids, especially in groups DHA and ALFA as well as in the EPA group.

In goat milk fat, between 1.06 and 1.73 wt % of the **palmitoleic** acid (16:1, n-7) was determined. There were no differences in the level of this fatty acid among groups before the supplementation with fatty acids. Among groups EPA, ALFA, and KONT, no statistically significant differences in the content of the palmitoleic acid in milk fat were observed neither during the supplementation with fatty acids nor after that. The content of palmitoleic acid in DHA group increased statistically significant (from 1.30% to 1.70%) from the second day of the supplementation with fatty acids. The high level of this fatty acid lasted till the ninth day after the supplementation ($p < 0.001$). The supplementation with unprotected n-3 fatty acids in cows reduced the content of palmitoleic acid in milk fat (Chilliard et al., 2001), what is contrary to our results.

Stearic fatty acid (18:0) in the goat milk fat was presented in the level from 2 to 14 wt %. There were no differences in the stearic acid content among groups before supplementation with fatty acids. Differences appeared during the supplementation with fatty acids, which were expressed the most in DHA group, where the percentage of stearic fatty acid fell from about 10 to less than 3% ($p < 0.001$). The fall of stearic acid during the supplementation with fatty acids was detected also in EPA group ($p < 0.05$), which was somewhat less pronounced, and the level of stearic acid re-established to the previous level within two days after the end of the supplementation with fatty acids. The previous level of stearic acid in DHA group was re-established five days after the end of supplementation with fatty acids. In ALFA and KONT group, there were no statistically significant differences in the levels of stearic fatty acid throughout the experiment. This information is a further indication, that the biodegradation of long-chain fatty acids (DHA) does not expire until the stearic acid, but there are several isomers of conjugated cis- and trans- C 18:2 fatty acids (Gulati et al., 1997; Gulati et al., 2000).

The content of **oleic** fatty acid (18:1, n-9) was in our experiment determined in the concentration from 19.0 to 28.0 wt %. During the supplementation with fatty acids, the content of oleic acid statistically significantly declined in EPA and DHA groups. An increase of the content of oleic acid in milk was observed in groups KONT and ALFA, as during as well as after the supplementation with fatty acids, but differences in these two groups before and after the supplementation were not statistically significant. Sanz Sampelayo et al. (2002) noted the content of oleic acid in goat milk around 22 to 24% and stated that despite the addition of various concentrations of protected polyunsaturated fatty acids the content of oleic fatty acid in goat milk remained fairly constant.

Conjugated linoleic acids (CLA) are a family of at least 28 isomers of linoleic acid found mainly in the meat and dairy products derived from ruminants. Several names could be

found for conjugated linoleic acid, most often conjugated linoleic acid, then rumenic or ruminal acid or *cis*-9, *trans*-11 octadecadienoic acid. It is one of those found only in ruminants and is a product of incomplete hydrogenation of fatty acids in the rumen (Clegg et al., 2001; Chouinard et al., 1999). In goats fed with fish oil (Gulati et al., 2000) mainly vaccenic fatty acid is formed due to the altered pattern of the biohydrogenation. In our experiment (Figure 2), goat milk of all observed groups contained less than 1.0% of the conjugated linoleic acid before the supplementation with fatty acids. During the second period, groups EPA, ALFA, and DHA statistically significantly differed ($p < 0.05$) from KONT group. The largest increase of the conjugated linoleic acid content during the supplementation appeared in DHA group, to over 3.0%. The content of conjugated linoleic acid in EPA group increased to 2.0%, and in ALFA group to 1.5%. The effect of the conjugated linoleic acid in DHA group was detected ten days after the supplementation with fatty acids. In nature, the most of the conjugated linoleic acids have their origin in alpha linolenic acid (Gulati et al., 2000), while in our experiment, the conjugated linoleic acid increased the most after feeding goats with docosahexaenoic acid (group DHA). Chilliard et al. (2001) fed cows with 200 to 300 g of the fish oil daily where the content of the conjugated linoleic acid increased from 0.2 to 0.6% to 1.5 to 2.7%. Authors mentioned that mainly rumenic acid increased which is presented also in our results, whereas the vaccenic acid occurred only in trace amounts and only a short time so that the findings published by Gulati et al. (2000) we could not confirm.

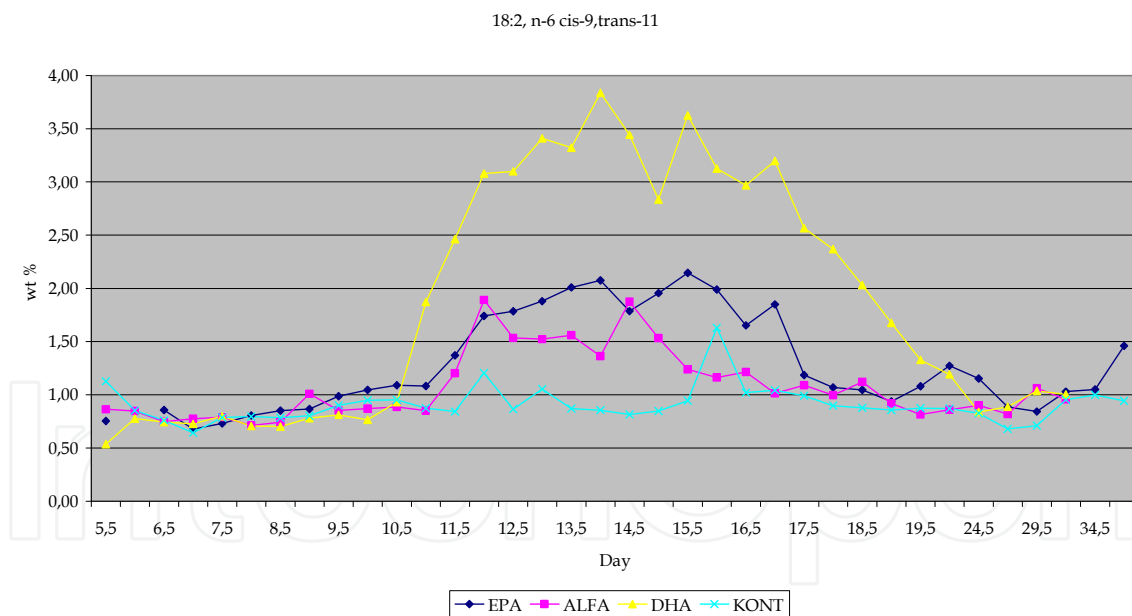


Figure 2. Average value of rumenic acid in goat milk

Conjugated linoleic acid is an intermediate product of the biohydrogenation, therefore its high concentration in DHA group was logical, since the degradation of the docosahexaenoic acid in the rumen is the slowest. The concentration of the conjugated linoleic acid in goat milk fat was relatively high also in ALFA group, knowing that the biohydrogenation of the α -linolenic acid is the fastest (Gulati et al., 1999), what we also observed in an increased concentration of C 18:1 in ALFA group. The conjugated linoleic acid is synthesised in the

mammary gland of lactating animals and in the muscles of young animals. In our experiment, the conjugated linoleic acid probably did not originate only from the supplemented fatty acids, what was found also by Griinari et al. (2000).

Before the supplementation with fatty acids, there was from 2.00 to 2.66 wt % of the **linoleic** acid (18:2, n-6) determined in goat milk fat in all groups. During the supplementation, the percentage increased in EPA group to 2.92% and in ALFA group to 3.4% ($p < 0.001$). Three days after the end of supplementation, the percentage dropped back to the previous value. There were no changes in the content of linoleic acid during the whole experiment in DHA and KONT groups.

α -linolenic (18:3, n-3 or octadecatrienoic) acid in goat milk was found in 0.50 to 1.00 wt %. During the supplementation with fatty acids, the percentage of α -linolenic acid increased only in the ALFA group to 3.20% and it dropped back to the previous level 0.50% ($p < 0.001$) four days after ending the supplementation. Thus, goats can successfully build linolenic fatty acid into milk fat when they are supplemented with this fatty acid.

There was less than 0.06 wt % of the **γ -linolenic** or cis-6,9,12-octadecatrienoic acid (18:3, n-6) in goat milk fat in all observed groups at the beginning of the experiment. After the addition of fatty acids into the goat diet, the content of the γ -linolenic acid increased in EPA group to 0.18%, in DHA group to 0.20% ($p < 0.05$), while the maximum increase to 0.33% appeared in ALFA group ($p < 0.001$). The increased content reflected three days after the end of supplementation with fatty acids and then decreased to the started value. Thus, γ -linolenic fatty acid is also successfully transferred into the milk fat, the fastest from α -linolenic fatty acid.

The content of **cis-11,14,17-eicosatrienoic** acid (20:3, n-3) in goat milk fat at the beginning of the experiment was 0.02 to 0.04 wt %. During the supplementation with fatty acids, the content increased only in the EPA group to 0.43% ($p < 0.001$). The content did not statistically significantly change in the other three groups. It is obviously, that eicosapentaenoic fatty acid was formed as a product of biohydrogenation, which occurred as an intermediate product only in milk fat of the EPA group.

At the beginning of the experiment, the content of **cis-8,11,14-eicosatrienoic** acid (20:3, n-6) was 0.02 to 0.03 wt %. During the supplementation with fatty acids, a slight increase of the content of cis-8,11,14-eicosatrienoic acid in DHA group to 0.04 to 0.05% and in EPA group to 0.08% was detected. Statistically significant increase of the cis-8,11,14-eicosatrienoic acid in goat milk fat occurred only in EPA group, from the third to the fifth day of the supplementation ($p < 0.05$). Immediately after ending the supplementation, the percentage of the cis-8,11,14-eicosatrienoic acid decreased in all observed groups to the value before the supplementation.

Arachidonic acid (20:4, n-6) was found in goat milk fat at the beginning of the experiment on average 0.20 wt %. During the supplementation with fatty acids, the percentage increased to 0.40% in EPA group and even to 0.60% in DHA group. Three days after ending the supplementation, the content of arachidonic acid in EPA group decreased to its starting level, while in DHA group, the content of arachidonic acid decreased after five days after the

end of the supplementation. The statistically significant increase in arachidonic acid content during the supplementation with fatty acids occurred in EPA and DHA groups ($p < 0.05$).

Eicosapentaenoic acid (20:5, n-3 or EPA) was determined in the goat milk fat at the beginning of the experiment in the content from 0.10 to 0.25 wt %. During the supplementation with fatty acids, the percentage changed in DHA group to 0.50 to 0.69%, while in EPA group the percentage rose to 2.00 to over 3.23%, as shown in Figure 3. Results showed that the level of eicosapentaenoic acid increased more than 30-times in milk, when animals consumed the eicosapentaenoic acid in the diet ($p \leq 0.001$). Statistically significantly higher content of eicosapentaenoic acid was observed in goat milk fat also five days after the end of supplementation, but only in EPA group.

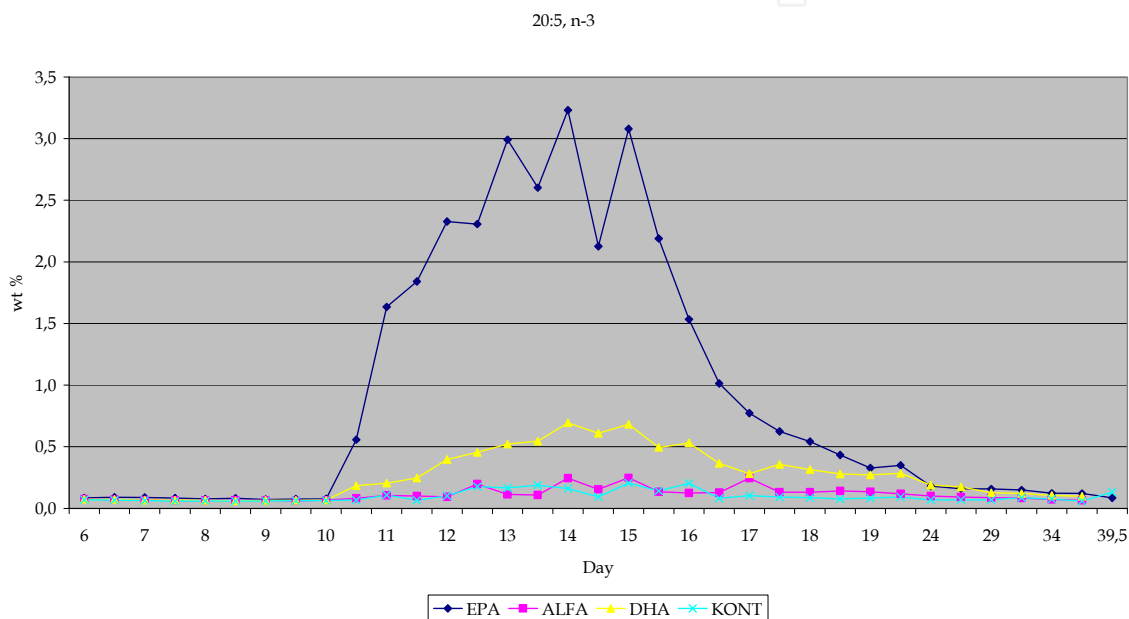


Figure 3. Average value of cis-5,8,11,14,17-eicosapentaenoic acid in goat milk

The maximum concentration of **eicosapentaenoic acid** was found on the fourth day of the supplementation with fatty acids, while Kitessa et al. (2001) noted the maximum on the sixth day, but they added only 160 mg of eicosapentaenoic acid per day as unprotected supplement, which was 125-times lower than in our case. Chilliard et al. (2001) stated the efficiency of transfer of the unsaturated fatty acids into cow's milk. The transfer was 2.6% for the eicosapentaenoic acid into cow's milk. In goats fed unprotected fatty acids, the transfer was 3.5% and 7.6% in goats fed protected fatty acids (Kitessa et al., 2001). The transfer of the eicosapentaenoic acid in our experiment was 7.1%, what had probably several reasons. The first reason can be relatively large dose of the supplemented eicosapentaenoic acid, the second short-term administration, whereas the ruminal microflora could not adapt for biohydrogenation of the eicosapentaenoic acid in this short time, and third, that according to the method of administering fatty acids the eicosapentaenoic acid partially passed through the rumen over esophageal gutter directly into the stomach.

According to the fact that the transfer of eicosapentaenoic acid through diet into the milk can be so effective, it is important how to produce milk enriched with n-3 and n-6 fatty

acids. Consumers are increasingly use milk with lower fat content. Thus, milk enriched with n-3 and n-6 fatty acids would significantly help to more correct and balanced diet, especially in children and elderly people.

Before supplementation with fatty acids, the content of **docosatrienoic** fatty acid in goat milk fat (22:3, n-3) was in all groups below the detection limit. During the supplementation, the increased content of docosatrienoic fatty acid was detected in EPA group, 0.03 to 0.06 wt %, and in DHA group, 0.6 to 0.11 wt % ($p < 0.001$). The increased value of the docosatrienoic fatty acid lasted until the 18th day of the experiment, and then it fell again below the detection limit. The value of the KONT group and ALFA group was below the detection limit through the whole time of experiment.

There was from 0.046 to 0.136 wt % of the **docosatetraenoic** fatty acid (22:4, n-6) in goat milk fat. During the supplementation, a slight increase of the docosatetraenoic fatty acid in EPA and DHA groups was noticed, but differences between groups in different periods of the experiment were not statistically significant.

Docosapentaenoic fatty acid (22:5, n-3) in goat milk fat was found in the concentration from 0.15 to 0.22 wt %. During the supplementation with fatty acids, the percentage of docosapentaenoic fatty acid increased in DHA group to 0.59% and in EPA group to 0.85% ($p < 0.001$). In both groups, an increased concentration of docosapentaenoic fatty acid reflected still 15 to 20 days after the end of the supplementation. The concentration was statistically highly significantly greater than the ALFA and KONT groups. It looks like docosapentaenoic fatty acid passes into the udder directly by blood, as it is not produced in the mammary gland de novo.

Only 0.05 to 0.1 wt % was the concentration of **docosahexaenoic** (22:6, n-3 or DHA) fatty acid in goat milk fat at the beginning of the experiment. During the supplementation with fatty acids, the percentage increased only in DHA group to 2.80%, and after the end of supplementation, it gradually declined. Even nine days after the end of supplementation with fatty acids, milk fat contained more than 0.50% of docosahexaenoic fatty acid (Figure 4). There was 3 to 4-times higher content of docosahexaenoic fatty acid in DHA group than in other groups ($p < 0.001$) even 20 days after the supplementation. The maximum concentration of docosahexaenoic fatty acid in goat milk fat in our experiment was found on the fifth day, while Kitessa et al. (2001) found the maximum concentration on the sixth day, but they added only 580 mg of docosahexaenoic fatty acid per day as an unprotected supplement, which is 34.5 times less than in our experiment.

The effectiveness of transfer the docosahexaenoic fatty acid into milk was observed in cows by Chilliard et al. (2001), which amounted 4.1%. In goats, it amounted 3.5% for unprotected fatty acids and 7.6% for protected fatty acids (Kitessa et al., 2001). The estimated transfer of docosahexaenoic fatty acid in our experiment was 7.84.

There was 53 to 57 wt % of the **medium chain** fatty acids in goat milk fat before the supplementation with fatty acids. After the supplementation, a decrease of the medium chain fatty acids was noticed in EPA, DHA, and ALFA group to 46 to 50%. The level of

medium chain fatty acids re-established to the starting level in three days after ending the supplementation in EPA and ALFA groups and in ten days in DHA group ($p < 0.05$).

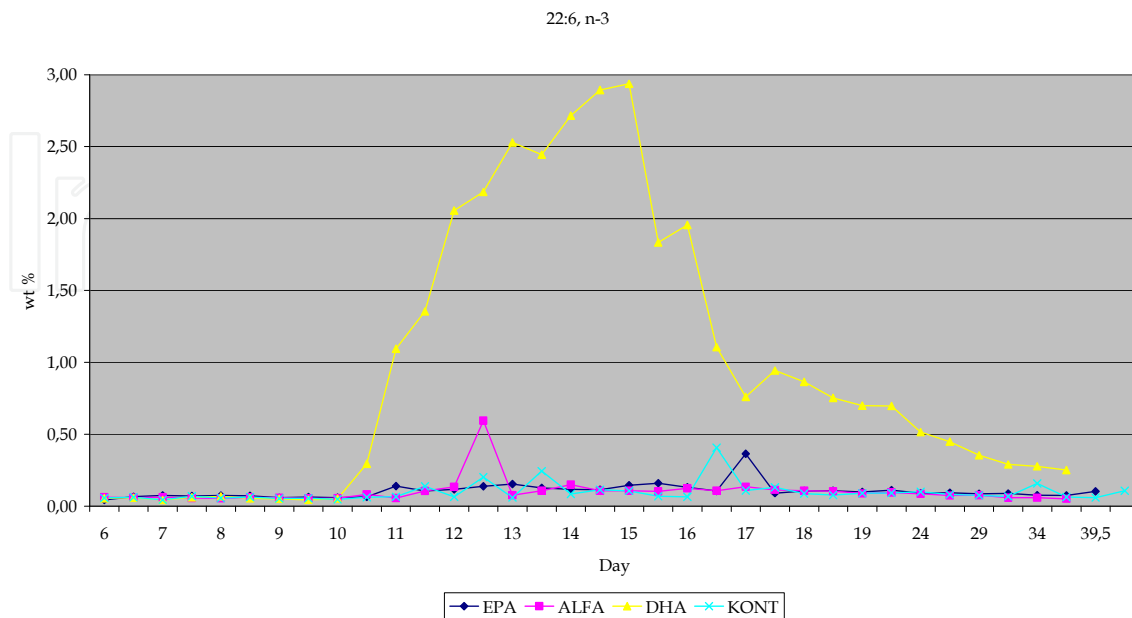


Figure 4. Average value of cis-4,7,10,13,16,19- docosahexaenoic fatty acid in goat milk

As reported Kitessa et al. (2001), a significant decrease appeared in C10 to C16 fatty acids after adding fish oil into the diet for goats, but when Chilliard et al. (2001) fed cows with fish oil only, they noticed a slight decrease in C4 to C14 fatty acids, or even 1.3% increase of these fatty acids when adding fish oil in the duodenum. In the experiment by Kitessa et al. (2001), a group of animals were supplemented a protected fish oil from 19th to 26th day and then unprotected fish oil from the 37th to 42nd day. Due to the significantly reduced feed intake and milk production in sheep the unprotected fish oil was administered a short time. Between one and another type of feeding was only eight days, which is questionable. It is possible that there was an influence of the previous supplementation, because our data showed that the effect of supplementation with some types of fatty acids can take more than 10 days on changes in the fermentation of medium chain fatty acids. Even Sanz Sampelayo et al. (2002) in goats found that the percentage of total unsaturated fatty acids reduced after the supplementation with protected polyunsaturated fatty acids.

The content of **monounsaturated** fatty acids in goat milk fat in our experiment ranged from 23 to 28 wt %, which reduced during the supplementation with fatty acids to 22% in EPA group and to 21% in DHA group. The decrease was statistically significant ($p < 0.05$) during the supplementation in EPA and DHA groups, while the reduction of monounsaturated fatty acids did not occur in ALFA and KONT groups. As reported Sanz Sampelayo et al. (2002), the supplementation of 9% polyunsaturated fatty acids only slightly increased the content of monounsaturated fatty acids, while the supplementation of 12% polyunsaturated fatty acids significantly increased the content of monounsaturated fatty acids.

Before the supplementation with fatty acids, **polyunsaturated** fatty acids were found in goat milk fat in the concentration from 4 to 6 wt %. The same level of polyunsaturated fatty acids

stayed in ALFA group throughout the whole time of experiment. A statistically significant ($p=0.001$) increase of the polyunsaturated fatty acids concentration appeared during the supplementation with fatty acids in EPA group (to 11%), ALFA group (9 to 10%), and in DHA group (11 to 11.9%). The peak in concentration of polyunsaturated fatty acids was achieved in EPA and DHA group on the fourth and fifth day of the supplementation and in ALFA group on the third day of the supplementation. The increased percentage of polyunsaturated fatty acids in goat milk fat persisted from 10 to 14 days in EPA, ALFA, and DHA groups.

The passage of the supplemented polyunsaturated fatty acids from the gastrointestinal tract into milk was estimated on the basis of the differences between the content of fatty acids before supplementation and the difference between KONT group and other groups during the supplementation and thereafter, taking into account the amount of milked milk during the supplementation and 14 days thereafter. The results are shown in Table 3, where it is clear that the passage of the conjugated linoleic acid into milk was 12.79%, 14.03% of the eicosapentaenoic acid, and 21.13% of the docosahexaenoic acid. The differences were statistically significant ($p < 0.05$).

GROUP	EPA	ALFA	DHA	KONT
Supplement	EPA	CLA	DHA	no
Supplemented PUFA during experiment (g)	95	72	75	0
PUFA appeared in milk from the 6 th to the 34 th day (g)	50.78	46.66	53.3	37.45
Difference or estimated passage (%)	14.03	12.79	21.13	0

EPA – eicosapentaenoic acid; CLA – conjugated linoleic acid; DHA - docosahexaenoic acid; PUFA –polyunsaturated fatty acids

Table 5. Estimated passage of the supplemented polyunsaturated fatty acids from food into milk

The **ratio between n-3:n-6** fatty acids before the supplementation with fatty acids was the same in all groups (1:3.50) and remained unchanged throughout the experiment only in KONT group. In all other groups, the ratio reduced during the supplementation with fatty acids to 1:1 and even to 1:0.67. It was gradually establishing back more than 20 days after the end of the supplementation. The differences before and after supplementation were statistically highly significant ($p < 0.001$).

3.3. Correlations between somatic cell count and some fatty acids

Correlations between somatic cell count and some fatty acids during the experiment were calculated by the Pearson correlation coefficient. The same correlations were calculated also for the second and third period of the experiment (from the 11th to the 65th day) and for the period from the 21st to the 65th day of the experiment. Statistically significant correlations between somatic cell count and C10 throughout the whole experiment were found in EPA group ($r=0.24$), ALFA ($r=-0.18$), and ($r=-0.17$) KONT group. The correlations between somatic cell count and C12 and between somatic cell count and C14 were statistically significant

throughout the whole experiment only in EPA group ($r=0.25$ and $r=0.24$, respectively; $p<0.01$). From the 11th to the 65th day of the experiment, there were only correlations between somatic cell count and C10 in DHA group ($r=-0.30$), between somatic cell count and C12 in DHA group ($r=-0.37$), and between somatic cell count and C14 in ALFA ($r=0.26$) and DHA ($r=-0.29$) groups found statistically significant ($p<0.05$). From the 21st to the 65th day of the experiment, correlations between somatic cell count and C10 in EPA ($r=-0.45$) and DHA ($r=-0.46$) groups, between somatic cell count and C12 in EPA ($r=-0.43$), DHA ($r=-0.53$), and KONT ($r=0.39$) groups, and between somatic cell count and C14 in ALFA ($r=-0.59$), DHA ($r=-0.57$), and KONT ($r=0.44$) groups were statistically significant ($p<0.05$).

Correlation between somatic cell count and C18:1 was statistically significant only in EPA group ($r=-0.24$) throughout the whole experiment, in DHA group ($r=0.47$) from the 11th to the 65th day of the experiment, and in EPA ($r=0.42$), ALFA ($r=-0.49$), and DHA ($r=0.67$) groups from the 21st to the 65th day of the experiment. Between somatic cell count and C18:3, the correlation was statistically significant only in ALFA ($r=-0.43$) group from the 11th to the 65th day of the experiment. No correlations between somatic cell count and C20:4 throughout the whole experiment were statistically significant. There were only correlations between somatic cell count and C20:4 in EPA group from the 11th to the 65th day of the experiment ($r=0.36$) and from the 21st to the 65th day of the experiment ($r=0.66$) statistically significant.

Statistically significant correlation between somatic cell count and monounsaturated fatty acids throughout the whole experiment was found only in ALFA group ($r=-0.22$) and from the 11th to the 65th day of the experiment in DHA group ($r=0.50$). From the 21st to the 65th day of the experiment, this correlation was statistically significant in EPA ($r=0.43$), ALFA ($r=-0.50$), and DHA ($r=0.68$) groups. Between somatic cell count and polyunsaturated fatty acids, only the correlation in ALFA group from the 21st to the 65th day of the experiment was found statistically significant ($r=-0.49$).

4. Conclusions

Our research proved that the supplementation of fatty acids into the diet had no effect on daily milk yield of goats. In ALFA group, a statistically significant impact on the increase of the protein content in milk ($p<0.01$) during the supplementation and thereafter was observed. Fat content was increasing during the supplementation and thereafter in ALFA group, while in EPA and DHA groups, fat content significantly reduced during the supplementation with fatty acids ($p<0.001$) and a few days thereafter. This finding indicates that the supplementation with fatty acids (eicosapentanoic and docosahexanoic fatty acid) had a negative impact on the milk fat production. Lactose content did not change significantly during the supplementation and no differences were found among groups. Non-fat dry matter content was the highest in ALFA group, its increased value reflected even after the end of the supplementation with fatty acids.

The supplementation of α -linoleic fatty acid decreased somatic cell count in milk, even 30 days after the end of the supplementation. Statistically significant decrease of somatic cell

count, compared to the period prior to the supplementation, was appeared till the 29th day after the end of the supplementation ($p < 0.05$). The number of micro organisms in milk is the result of hygienic conditions at milking, hygiene of milking personnel, equipment, environment and hygiene of the animals. In the case of our study, it has been established that the lower number of micro organisms was the consequence of better hygiene during the experiment due to the experimentalists' presence.

The supplementation of α -linoleic, eicosapentanoic and docosahexanoic fatty acids had different effects on the composition of fatty acids in milk fat. Eicosapentanoic fatty acid supplemented into the diet of EPA group increased the following fatty acids: capric, lauric, myristic, conjugated linoleic, linoleic, γ -linolenic, cis-11,14,17-eicosatrienoic, cis-8,11,14-eicosatrienoic, arachidonic, eicosapentaenoic, docosatrienoic, docosatetraenoic, and docosapentaenoic acid. The supplementation of eicosapentanoic fatty acid decreased palmitic, stearic, and oleic fatty acid. α -linoleic fatty acid supplemented to ALFA group increased the following fatty acids: lauric, miristoleic, oleic, conjugated linoleic, linoleic, α -linoleic, γ -linolenic acid. This means that there was no elongation from α -linoleic acid into fatty acids with longer chain. A decrease was observed in myristic, palmitic, and docosatetraenoic acid. DHA group was supplemented with docosahexanoic fatty acid where the increase of the following fatty acids was recorded: capric, lauric, myristic, palmitoleic, conjugated linoleic, linoleic, γ -linolenic, cis-8,11,14-eicosatrienoic, arachidonic, eicosapentaenoic, docosatrienoic, docosatetraenoic, docosapentaenoic, docosahexanoic acid, while a decrease was noticed in the following fatty acids: miristoleic, palmitic, stearic, and oleic acid. In the control group, only slight variations in some fatty acid levels were recorded, which were not statistically significant.

Research showed that the supplementation of α -linoleic acid had a positive impact on reduction of the somatic cell count in goat milk. However, the surprising result was found, that the supplementation of eicosapentanoic and docosahexanoic acid did not affect the reduction of somatic cell count in the same extent. There is a question, whether this is the result of the supplement or of the n-3:n-6 ratio which changed after the supplementation. Since the ratio n-3:n-6 changed to the similar value when other fatty acids were supplemented and the effect was not the same, it seems that the n-3:n-6 ratio was not the cause of this effect. It is suggested that α -linoleic acid could be rapidly incorporated into cell membranes, which displace arachidonic acid. This is resulted in more flexible cell membranes and better anti-inflammatory effect. Perhaps this mechanism was the one which contributed to the reduction of somatic cell count. For further research, it would be necessary to also include this kind of analysis. The results also showed that the transition of long chained polyunsaturated fatty acids into goat milk appeared relatively in large extent, therefore, polyunsaturated fatty acids occur in milk fat very quickly after their consumption.

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