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FOOD ANALYSIS /
ANALIZA HRANE

COMPARATIVE ANALYSIS OF CONJUGATED LINOLEIC ACID CONTENT IN COW, SHEEP AND GOAT MILK

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ABSTRACT

In recent years there has been increased interest in research on conjugated linoleic acid (CLA) and its potential health benefits: anticarcinogenic, antiatherogenic, antidiabetic and immunomodulatory effects. Term CLA describes a group of positional and geometric isomers of linoleic acid characterized by conjugated system of double bonds, separated by one single bond. The objective of this study was to analyze the differences in fatty acid (FA) profiles in cow, sheep and goat milk with an emphasis on the content of CLA. Milk samples were collected at conventional cow, sheep and goat farms, and according milk type were grouped in three groups. FA profiles were analyzed by gas chromatography. FAs were analyzed as methyl esters (FAMEs), and identified by comparison with methyl esters of standards. According to obtained results, significant difference ($p<0.05$) in CLA content was noted between three groups of samples, and also considerable individual variations in CLA content were noted inside each of group. Sheep milk samples were richer in CLA compared to goat and cow's milk samples. Milk fat is the richest natural dietary source of CLA, and its content can be increased by manipulation of feeding regimes and genetic selection of dairy animals.

Keywords: CLA, fatty acids, GC-FID

INTRODUCTION

The changes in the alimentary patterns in the last years are the reasons for many human diseases. Lifestyle related diseases, such as obesity, hyperlipidemia, arteriosclerosis, diabetes mellitus and hypertension are increasingly prevalent in developed countries. The pathogenesis of lifestyle related deseases is complicated, and it is has been noted that dietary lipids could be important modulator in morbidity of these deaseases (Nagao and Yanagita, 2005).

In recent years there has been a growing interest in foods that contain components with bioactive properties. Milk and dairy products have long traditions in human nutrition (Miller et al., 2000; Rogelj, 2000).

The composition of milk is quite complex. Its components are subject of research for many years. Milk is composed of various substances with bioactive properties and therefore milk has been given an epithet of functional food. Not only nutritional value but also other physiological properties of milk components are subject of interest (Miller et al., 2000).

The most variable component of milk is milk fat. It is one of the components which determine nutritive quality and technological performances of milk. Milk fat has influence on smell and aroma of milk, and on consistence and texture of dairy products. Despite of other milk components milk fat has highest energetic value (9 kcal/g or 37 kJ/g).

Milk fat is a complex of lipids, and exists in microscopic globules in an oil-in water emulsion in milk. The majority of milk lipids are triglycerides or the esters of fatty acids combined with glycerol (97 – 98%), and the minority are phospholipids (0.2 – 1%), free sterols (0.2 – 0.4%) and traces of free fatty acids. About 62% of milk fat is saturated, 30% monounsaturated, 4% polyunsaturated and 4% of minor types of fatty acids (Miller et al., 2000; Jensen, 2002; Keenan and Mather, 2003; Evers, 2004). Milk fat plays an important role in the nutritional quality and technological properties of milk, and its composition is directly involved in the health of human consumers. It is one of the most complex natural fats that consist of approximately 400 – 500 fatty acids (Barłowska and Litwińczuk, 2009).

It contains a number of components which are metabolically active such as: sphingolipids, conjugated linoleic acids (CLA), butyric acid, other fatty acids, vitamins A and D. A variety of health benefits have been associated with these compounds (Bauman and Lock, 2010).

CLA which are naturally occurring fatty acids found in animal and dairy fats, exhibit a number of health benefits. CLA are found in relatively large quantities in the milk and/or meat of ruminant animals and appear to be metabolized differently than linoleic acid. In the diet of many consumers, meat and dairy products are a significant source of CLA (Barbosa et al., 2003).

CLA refers to a mixture of 28 positional and geometric isomers of linoleic acid (C18:2, *cis*-9, *cis*-12) with two conjugated double bonds at various carbon position in the fatty chain. The most abundant isomer in food products from ruminants is *cis*-9, *trans*-11 (rumenic acid) comprising 80 – 90% of the total CLA, whereas *cis*-12, *trans*-10 is present in smaller amounts (3 – 5%). Both isomers have been proven to have biological activities. CLA found in milk and meat of ruminants originates from two sources. It is formed as an intermediate during the biohydrogenation of linoleic acid by linoleic acid isomerase from the rumen bacteria *Butyrivibrio fibrisolvens* or from the endogenous conversion of C18:1 *trans*-11, (vaccenic acid) another intermediate of linoleic or linolenic acid by Δ9-desaturase in the mammary gland (Figure 1) (Griinari et al., 2000; Bauman et al., 2003; Dhiman et al., 2005).

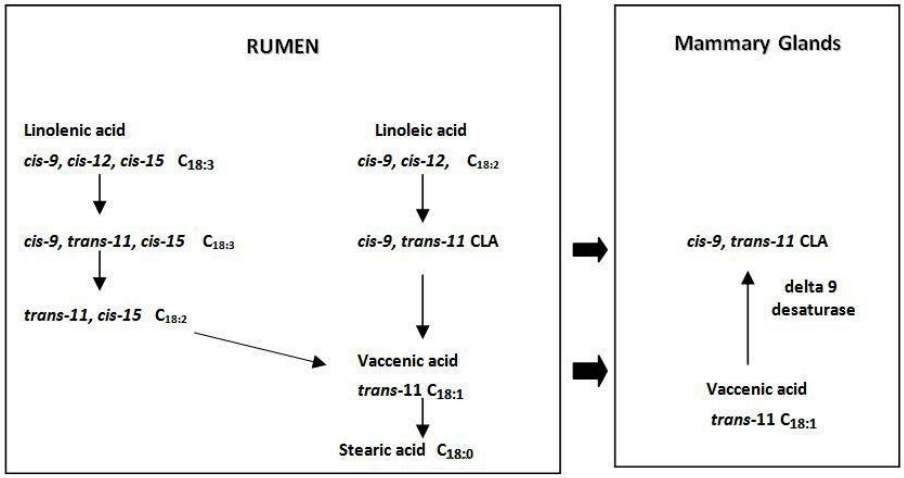

Figure 1. Metabolic pathways involved in the biosynthesis of CLA in ruminants (Bauman et al., 2003)

Milk fat and meat of ruminants are the richest natural dietary source of CLA. Reported beneficial health-related effects of CLA include: anticarcinogenic, antiatherogenic, antidiabetic and immune modulating properties. Hence CLA is considered as functional food (Akalin et al., 2006).
The objective of this study was to analyze the differences in fatty acid profiles in cow, sheep and goat milk with an emphasis on the content of CLA.

MATERIALS AND METHODS

Milk samples (n=60) were collected at conventional cow, sheep and goat farms belonging to three diferent breeders, and according the milk type (cow, sheep or goat milk) were grouped in three groups. Samples of raw milk from each of herd (cow, sheep, and goat) were collected in period of one month from (April to May) (n=20 samples per herd). Herds were grazing on pastures at highland area in Pelagonia region.
Extraction of milk fat was performed using 25% ammonia, 95% ethyl alcohol and hexane (Rose-Gottlieb, AOAC (2000), modified according Secchairi et al. (2003)). To minimize oxidative degradation of fatty acids the butylated hydroxytoluene was added as a preservative. Fatty acids were than trans-esterified with BF_3/Methanol into fatty acid methyl esters (FAMEs). Fatty acids methyl esters (FAMEs) were prepared according to AOAC Official Method 996.06 (2000). Analysis of the FAMEs was carried out on a GC-FID, (GC Agilent Technologies 7890 GC System, CN 11251075, USA). Column HP88 (J&W 112 -8867; 250 °C; 60m x 250mm x 0.2 mm, Agilent, USA) was used for FAMEs analysis. The injector and detector temperature were 250 °C and 300 °C respectively. The column temperature parameters were as follow: initial oven temperature of 70 °C (1 minute) was ramped at 5 °C/minute to 100 °C and maintained for 2 minutes. Than temperature was increased at 10 °C/minute to 175 °C and maintained for 2 minutes, which was

followed by last increasing at 3 °C/minute to 220 °C and hold for 5 minutes. Injection volume of FAMEs was 1 μL, helium was carrier gas, and total run was 38.50 minutes. The reliability and accuracy of the analytical method for the detection of fatty acids were ensured by use of the certified reference matrix that consisted a mixture of FAME standards (Supelco, Sigma-Aldrich). The calculation of the results was done with Chemstation software, and results were expressed as percentage of identified fatty acid on total analysed fatty acids (%).
Statistical analysis was performed using the statistical software package SPSS 15.0. The results are presented as the means ± SD. One way ANOVA was used to assess the statistically significant difference between groups of samples grouped according to milk type. The level of statistical significance was determined at $p<0.05$.

RESULTS AND DISCUSSION

Milk fatty acid composition depends on many factors, such as: genetic predisposition, stage of lactation, nutrition etc. The objective of this study was to analyze the differences in fatty acid profiles in cow, sheep and goat milk with an emphasis on the content of CLA.
According obtanied results (Table 1), comparative analysis of fatty acid profile of cow, sheep and goat milk has shown higher content of medium-chain fatty acids (C8:0 – caprylic acid, and more markedly, C10:0 – capric acid) in goat milk, compared with cow and sheep milk. These fatty acids reduce the production of cholesterol in the human body (Chilliard et al., 2006). Conversely, cow milk has higher content of butyric (C4:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0) and oleic (C18:1) acids compared with sheep and goat milk. In this respect, goat milk contains about 30% medium chain fatty acids (C6:0 – C14:0), cow milk about 21%, while sheep milk 25%.
Highest content of saturated fatty acids was noted in goat milk samples, then follow cow and sheep milk. Conversely, highest content of unsaturated fatty acids has cow milk, than follow samples of sheep and goat milk. The same pattern was noted in monounsaturated fatty acids (MUFA). The highest content of polyunsaturated fatty acids (PUFA), specially α-linoleic acid (C18:3 *cis*-9, *cis*-12; *cis*-15) was noted in sheep milk, compared with cow and goat milk.
The comparative analysis of CLA content between three groups of samples has shown significant difference ($p<0.05$). Sheep milk samples were richer in CLA than goat and cow's milk samples (Table 1).
In general, the results obtained from the analysis of the fatty acid profile of the three groups of samples are in accordance with the data of numerous studies. Fatty acid composition of milk fat is affected by several factors, such as: lacting ruminant`s species, breed, age, diet, and management factors related to feed supplements affecting the diet (Bauman and Griinari, 2001; Dhiman et al., 2005; Arnould and Soyeurt, 2009).

Table 1. Fatty acids profile in goat, sheep, and cow milk

Fatty acids (%)	Cow milk (n=20)	Sheep milk (n=20)	Goat milk (n=20)
C4:0; butiric acid	2.91 ± 0.63[a]	2.61 ± 0.67[a]	2.07 ± 0.35[b]
C6:0; caproic acid	2.05 ± 0.64[a]	1.91 ± 0.37[a]	2.82 ± 0.31[b]
C8:0; caprylic acid	1.43 ± 0.24[a]	1.95± 0.28[a]	2.96 ± 0.27[b]
C10:0; capric acid	3.07 ± 0.77[a]	6.67 ± 0.84[a]	9.83 ± 0.62[b]
C12:0; lauric acid	3.68 ± 0.91[a]	4.03 ± 0.74[a]	4.56 ± 0.71[a]
C14:0; myristic acid	10.96 ± 1.53[a]	10.21± 1.50[a]	9.87 ± 1.83[a]
C16:0; palmitic acid	28.74 ± 2.47[b]	25.14 ± 3.48[a]	24.68 + 1.4[a]
C18:0; stearic acid	11.27 ± 2.24[b]	8.89 ± 2.32[a]	8.91 ± 2.30[a]
C18:1, *cis*-9; oleic acid	22.40 ± 3.47[a]	20.22 ± 3.24[a]	18.69 ± 4.5[b]
C18:2, *cis*-9, *cis* 12; linoleic acid	2.61 ± 0.97[a]	2.36 ± 0.75[a]	2.29 ± 0.60[a]
C18:2, *cis*-9, *trans*-11; linoleic acid - CLA	0.61 ± 0.74[a]	0.80 ± 0.73[b]	0.49 ±0.57[a]
C18:3 *cis*-9, *cis*-12; *cis*-15; α-linoleic acid	0.54 ± 0.46[a]	0.96 ± 48[b]	0.81 ± 36[b]
SFA	64.11 ± 4.76[a]	61.41 ± 3.55[a]	65.70 ± 3.48[a]
UFA	26.16 ± 3.91[a]	24.34 ± 3.06[a]	22.28 ± 3.95[b]
PUFA	3.76 ± 0.95[a]	4.12 ± 1.37[b]	3.59 ± 1.07[a]

SFA – saturated fatty acids; UFA – unsaturated fatty acids; PUFA – polyunsaturated fatty acids
Different superscript letters in the same row indicate significant differences ($p<0.05$)

According literature data total CLA content in milk or dairy products ranges from 0.34% to 1.07% of total fat (Dhiman et al., 2005). Obtained vaules of CLA content in analysed samples of cow, sheep and goat milk are in accordance with literature data.

The animal diet strongly influence CLA content of milk (Dhiman et al., 2005). Literature data indicate that pasture based diets have positive effects of on the CLA content of milk (Kelly et al., 1998a; Kelly et al., 1998b; Dhiman et al., 2000; Ward et al., 2003). Collomb et al. (2002) have noted that variation in CLA content could be due to differences in fatty acid composition of the plant species in the pasture.

On the other hand, Dhiman et al. (2005) suggest the individual variation of CLA content in milk among lacting animals inside herds, even when the same diet is fed.

These differences could be due simply to differences in desaturase enzyme activities in the mammary gland, age of animals, disease conditions, differences in ruminal metabolism, or other factors.
Milk fat biosynthesis is a complex process, and acorrding Chilliard et al. (2006) the regulation in mammary cells differs between caprine and bovine species, particularly in the elongation process of FA, which are synthesized *de novo* by the "fatty acid synthase" complex.
According literature data it is widely recognized that diet plays a primordial role in modulating fatty acid composition of ruminants' milk (Jensen, 2002; Chilliard et al., 2006).
Analysis of factors affecting milk fatty acids composition is very important for understanding of physiological and biochemical characteristics of milk, which have impact on the maintenance or improvement of the consumer's health.

CONCLUSION

According obtained results sheep milk samples were richer in CLA than goat and cow milk samples. In recent years, conjugated fatty acids have attracted considerable attention because of their pottential beneficial effects of atenuating lifestyle related disease. Milk fat is the richest natural dietary source of CLA, and its content can be increased by manipulation of feeding regimes and genetic selection of dairy animals.

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