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The influence of the season on the fatty acid composition and conjugated linoleic acid content of the milk

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Abstract. The purpose of the research was to determine the fatty acid composition of milk of general varieties in Hungary that is Hungarian Simmenthal, Red Holstein Friesian and Black Holstein Friesian and the

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changes in the fatty acid composition of their milk fat throughout the year with special respect to the conjugated linoleic acid content. The amount of unsaturated fatty acids (oleic acid, linoleic acid and linolenic acid) including conjugated linoleic acid was higher in summer than in winter. In the case of the saturated fatty acids (butyric acid, caproic acid, caprylic acid, capric acid, myristic acid, palmitic acid and stearic acid) an opposite tendency has been shown. The amount of conjugated linoleic acid ranged from 0.8 to 1.4%, with an average value of 1.1%.

1 Introduction

Milk fat as a fat source for the human nutrition used to be regarded as harmful to health because it is a rich source of the saturated fatty acids. New scientific results have demonstrated that milk fat contains components with advantageous impact on health and these materials proved to be effective against cancer or atherosclerosis [4, 5, 6, 9, 10, 11]. Certain long chain unsaturated monocarboxylic acids that is conjugated linoleic acids (CLA) also belong to this group. CLA means several positional and geometrical isomers of octadecadienoic acid with the two double bond in the conjugated position. When the positive effect of CLA on health was proven by many researcher, the question arose how to increase the CLA intake of human. The CLA content of different sort of foods has been surveyed and now the factors that exert an effect on the CLA concentration of food is under studying. On the one hand CLA can be originated from the processes of biological hydrogenization occurs in the rumen of cow and other ruminants, on the other it can be formed during the heat processing of food.

In the case of milk fat $c9$,$t11$-C18:2 ($c9$,$t11$-CLA) is the main component giving more than 80% of the total CLA content [1, 3, 12]. The CLA content of the milk fat has been shown to vary from 0.2 to 2.0 g CLA/100 g milk fat. In Sweden the CLA content of the milk fat was found between 0.25 and 1.77 g CLA/100 g milk fat [8], in the EU the average amount of $c9$,$t11$-CLA was 0.76 g CLA/100 g milk fat and the extreme values were 0.13 and 1.89 g CLA/100 g milk fat [13]. Among the factors that affects the CLA content of milk the influence of season and housing can be attributed to the different feeding techniques. The most important factor connected with feeding is the unsaturated fatty acid content of the fodder, that means mostly linoleic acid and linolenic acid content, because these fatty acids are supposed to be the precursors of CLA.

The first author was *Riel* [14] who pointed out that the CLA content of

milk in summer had been twice as high (1.46%) than in winter (0.78%) based on the results of spectrofotometric determinations. This finding was supported by others who mesaured significantly higher CLA content in milk from cows drown up to the pasture than cows feeding with hay and/or silage using chromatographic methods [2]. An experiment on the milk of cows from the EU countries has shown that the poliunsaturated fatty acid (PUFA) intake of animals grazing on the pasture is higher than that of animals kept in the cow-sheed and fed with preserved fodders [13]. The type of the farming (conventional or ecological) has also an effect on the CLA content of milk [7]. The amount of CLA ranged from 0.34 g CLA/100 g milk fat (animals kept in cow-sheed) to 0.8 g CLA/100 g milk fat (animals kept in ecological farms).

The CLA content of milk of cow and the changes in the CLA content of milk fat throughout the year has not been determined yet in Hungary. The purpose of the present research was to determine the fatty acid composition of milk of general varieties in Hungary and the changes in the fatty acid composition of milk fat with special respect to CLA content. The present work is supposed to be a preliminary research with limited number of repetition in order to establish a higher volume research involving higher number of individuals and repetitions.

2 Materials and methods

2.1 Animals and feeding

The milk samples were collected from the selected individuals of 210 cows throughout a year at the dairy plant called "Új Élet" at Hencida. Half of the cows were Black Holstein Friesian 15% of them Red Holstein Friesian and 30% was the ratio of Hungarian Simmenthal. During the summer period (from 10^{th} May until 15^{th} October) the animals were kept on the pasture and the grass was supplemented with 3.5 kg of fodder that contained 20% dairy concentrate 60% corn and 20% wheat. The diet was supplemented with phosphorus and calcium and 10–15 kg corn silage. During winter alfalfa and grass hay were supplied ad libitum and the diet consists of 3.5 kg of dairy forage, mineral supplementation, 15 kg of slice of sugar-beet and 15 kg of corn silage.

The bulk milk of the individual cows was sampled and in the case of each variety three milk samples were drown that means 100 ml of milk. The samples were immediately cooled down and stored frozen at $-25\,^{\circ}\text{C}$ until the chemical analysis.

2.2 Chemical analysis

Determination of fatty acid composition: The laboratory analysis of samples was carried out at the Department of Chemistry and Biochemistry, Faculty of Animal Science, University of Kaposvár. The homogenized sample was weighed into a flask, 8 ml concentrated hydrochloric acid was added and it was boiled for 60 minutes. After cooling down 7 ml ethanol was added then 15 ml diethylether following one-minute-shaking. The next extraction was with 15 ml petrolether (b.p.<60 °C). After phase separation organic phase that contains about 150–200 mg fat was separated and evaporated under vacuum on a rotadest. Then 4 ml 0.5 M sodium-hydroxide in methanol was added, and boiled on a water bath for 5 minutes. Then 4 ml 14% boron-trifluoride in methanol was added and boiled for 3 minutes following the addition of 4 ml n-hexane. It was boiled for one minute then the level of the organic phase was brought to the neck of the flask with saturated sodium-chloride solution. When phases were separated samples were taken for the analysis from the organic phase, and it was dry on sodium sulfate.

The fatty acid methyl esters (FAMEs) were separated on a 100 m×0.25 mm wall coated open tubular (WCOT) column equipped with CP-SIL 88 (FAME) stationary phase. The quantitation of FAMEs was obtained with a flame ionization detector (FID) at 270 °C. The temperature of the splitter injector was 270 °C, the carrier gas was helium with the head pressure of 235 kPa. The oven was temperature programmed from 140 °C (10 min.) with 10 °C/min increase up to 235 °C (26 min). The injected volume varied between 0.5 and 2 μl. The instrument was a Chrompack CP 9000 gas chromatograph.

Determination of conjugated linoleic acid content: 10 ml milk sample was weighed and 80 ml organic solution mixture (a mixture of hexan:i-propanol in the ratio of 3:2, HIP) was added. The sample was dispersed with an IKA Ultra-turrax dispersion instrument and filtered. The filtration apparatus was rinsed three times with 10 ml HIP. The water was eliminated from the organic phase with the addition of 5 g anhydrous sodium sulphate. The HIP mixture was evaporated to dryness from the lipids and the residue was washed with hexane into a volumetric flask. Transesterification (methylation) of lipids was accomplished with 4 M sodium methylate solution in methanol. The reaction was completed at 50 °C for 30 minutes. The resulting FAMEs were extracted with hexane and injected into the same column as was used in the case of the determination of the other FAMEs. The oven was kept at 140 °C for 10 min and the temperature was raised with 5 °C/min until 235 °C then held

for 30 minutes.

3 Results

The changes in the fatty acid composition and CLA content in the function of the months can be seen in *Table 1.* The results are given as the averages of the three varieties. Among the isomers of CLA the $c9,t11$-C18:2 isomer is present at the highest amount in the milk fat and has been reported to has health protecting role, therefore the amount of this isomer was determined. *Figures from 1 to 5* show the changes in the ratio of different fatty acids of milk fat. The milk of different genotypes is assigned with various lines. Due to the limited financial opportunities there were only three analyses per variety and per samples. The mean of the three analyses can be seen in *Table 1.* The absence of standard deviation values and statistical analysis is due to the limited number of repetition.

Figure 1: The butyric acid (C4:0) and caproic acid (C6:0) content of milk fat in the function of months expressed in the relative weight-percentage of the fatty acid methyl esters

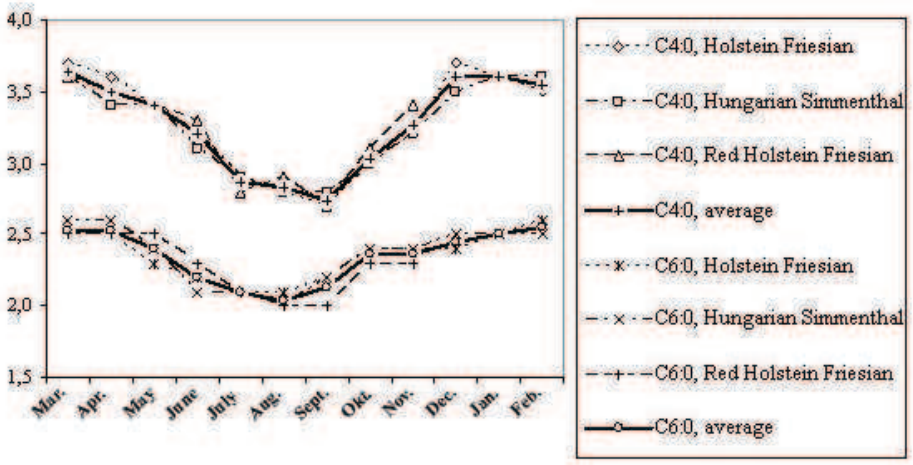

Figure 2: The caprylic acid (C8:0) and capric acid (C10:0) content of milk fat in the function of months expressed in the relative weight-percentage of the fatty acid methyl esters

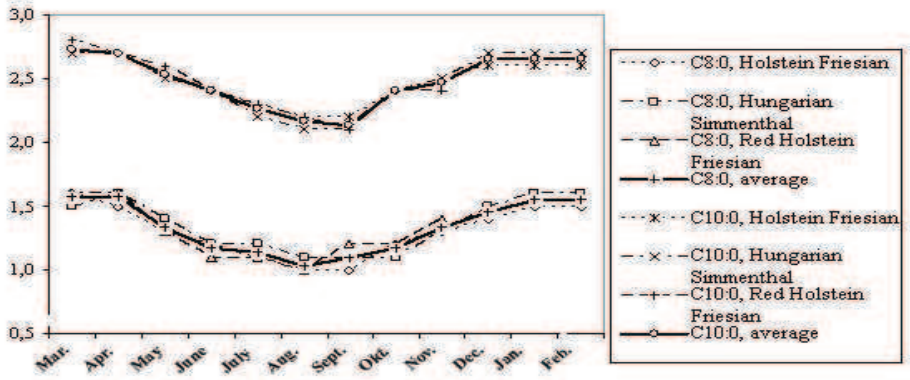

Figure 3: The palmitic acid (C16:0) and oleic acid (18:1) content of milk fat in the function of months expressed in the relative weight-percentage of the fatty acid methyl esters

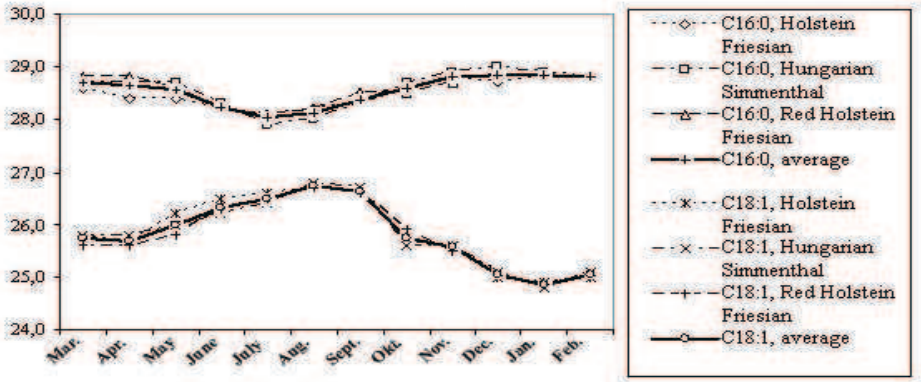

Figure 4: The linoleic acid (C18:2) and linolenic acid (C18:3) content of milk fat in the function of months expressed in the relative weight-percentage of the fatty acid methyl esters

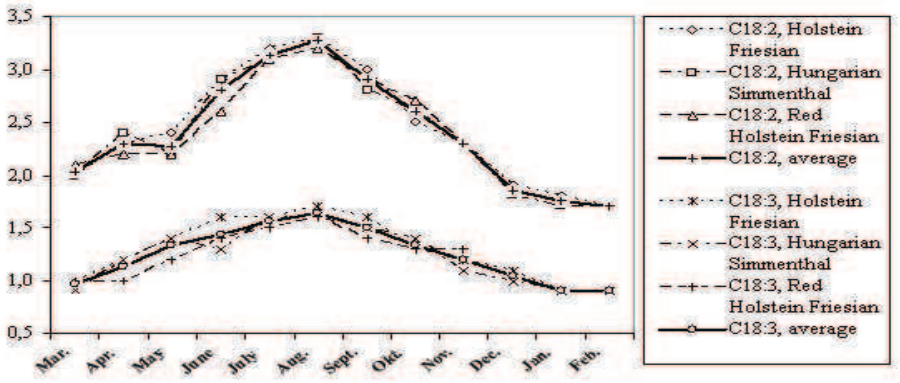

Figure 5: The conjugated linoleic acid content of milk fat in the function of months expressed in the relative weight-percentage of the fatty acid methyl esters

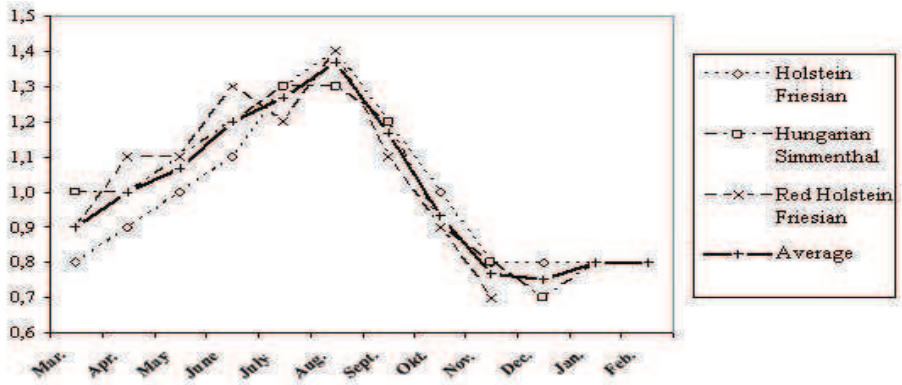

Table 1: The average fatty acid composition of the milk fat of three genotypes expressed in the relative weight-percentage of the fatty acid methyl esters

	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Butyric acid C4:0	3.6	3.5	3.4	3.2	2.9	2.8	2.8	3.0	3.2	3.6	3.6	3.5
Caproic acid C6:0	2.5	2.5	2.3	2.2	2.0	2.1	2.2	2.4	2.4	2.4	2.5	2.6
Caprylic acid C8:0	1.6	1.5	1.4	1.2	1.2	1.1	1.0	1.2	1.3	1.4	1.5	1.5
Capric acid C10:0	2.7	2.7	2.5	2.4	2.3	2.1	2.1	2.4	2.5	2.6	2.7	2.7
Lauric acid C12:0	3.4	3.2	3.3	3.3	3.3	3.2	3.4	3.4	3.4	3.5	3.5	3.4
Myristic acid C14:0	11.1	11.0	11.1	10.9	11.0	11.0	11.3	11.4	11.4	11.5	11.7	11.6
Myristoleic acid C14:1	1.4	1.3	1.2	1.3	1.5	1.4	1.5	1.6	1.5	1.6	1.6	1.6
Pentadecanoic acid C:15:0	1.2	1.1	1.1	1.0	1.1	1.2	1.1	1.2	1.1	1.2	1.2	1.2
Palmitic acid C16:0	28.6	28.6	28.5	28.5	28.0	28.1	28.3	28.7	28.8	28.7	28.9	28.8
Palmitoleic acid C16:1	2.6	2.6	2.5	2.5	2.4	2.3	2.4	2.5	2.6	2.6	2.6	2.6
Heptadecanoic acid C17:0	1.0	1.1	1.1	1.0	1.1	1.0	1.1	1.2	1.3	1.3	1.3	1.3
Stearic acid C18:0	10.6	10.6	10.6	10.6	10.4	10.4	10.5	10.5	10.6	10.7	10.7	10.8
Oleic acid C18:1	25.8	25.9	26.2	26.3	26.7	26.9	26.5	25.7	25.6	25.1	24.7	25.0
Linoleic acid C18:2	2.0	2.3	2.4	2.9	3.2	3.3	3.0	2.5	2.3	1.9	1.8	1.7
Linolenic acid C18:3	1.0	1.2	1.4	1.6	1.6	1.7	1.6	1.3	1.2	1.1	0.9	0.9
CLA c9,t11-C18:2	0.9	0.9	1.0	1.1	1.3	1.4	1.2	1.0	0.8	0.8	0.8	0.8

The figures show that the fatty acid composition of the milk fat of the three varieties is almost the same and their changes in the function of the season did not depend on the sort of the variety. Based on the results of this preliminary research the difference between varieties is negligible. It seems to be not probable that with the increase of the number of individuals significant

differences among varieties could be shown. Higher variation was observed in the case of CLA among fatty acids, but this is not connected with the difference of varieties. It can be connected with the uncertainty of the analytical method and the seasonal changes in the composition of forage. The absolute value of the standard deviation is not higher that in case of the other fatty acids, but due to the minor amount of CLA in milk fat, the relative standard deviation is higher. However, the means of the different varieties are almost the same.

When the different fatty acids are evaluated individually, it can be concluded, that the butyric acid has a minimum level of 2.6–2.8% between June and September, and a maximum value of 3.5–3.7% between December and April. The changes in the ratio of caproic acid, caprylic acid and capric acid have a similar tendency in the function of the months like butyric acid. They dropped to their minimum levels between July and September, and reached their maximum values during the winter and early spring months. The minimum level of caproic acid is 2.1–2.2% in August and in September, the maximum value is 2.6–2.7% between December and April. Among the short carbon chain fatty acids the concentration of caprylic acid is the lowest in the milk fat of cows under study. The lowest concentration was between 1.1 and 1.2% measured between July and September and the highest amount of it was 1.6% between January and April. Among the fatty acids of the milk fat palmitic acid and oleic acid are present in the highest quantities. The changes in the percentage ratio of palmitic acid throughout the year is very similar to the tendency that the short chain fatty acids show, its minimum value is 28.1–28.3% between July and August, the maximum level is 28.7–29.0% during the winter and the early spring months.

The changes in the ratio of the unsaturated fatty acids in the milk fat show an opposite tendency throughout the year related to that of saturated fatty acids. The oleic acid, that is present in the milk fat of the second highest concentration, has the highest concentration of 26.5–26.7% between July and September, and its amount dropped to 25.0% during winter months. Regarding the effect of the season, linoleic acid and linolenic acid show similar tendency than oleic acid, namely both polyunsaturated fatty acids reach their maximum between July and September. The milk fat during summer contains 3.2–3.3% of linoleic acid and its amount in winter is 1.7–1.8%. In August the linolenic acid has a maximum with 1.6% that is dropped to 0.8–0.9% during the winter and the early spring month. The CLA content of milk fat is the highest in August that is 1.35% calculated as the average value of the varieties. Between July and September the CLA content of milk fat of each variety exceeds 1.2% and this value rapidly decreases during the autumn month and

drops to 0.75–0.80% during winter months. These results are in agreement with the statements of Riel [14] that the CLA-content of raw milk is twice as high in summer than in winter, but in the present work the difference between the two extreme values was smaller (from 0.8 to 1.4%). Our findings are similar to that of Dhiman et al. [2] that is the CLA content of the milk increases when the cows are driven out to the pasture. The CLA content of milk fat in the case of cows were kept in the cow-sheed during winter was measured 2–2.5 times higher than Jahreis and coworkers observed [7] and the CLA-values measured during the summer were also higher than that of measured on an ecological farm. The range between the extreme values of CLA (0.8–1.4%) was smaller than in the other authors' experiments (0.2–2.0 CLA/100 g milk fat, Chin et al. [1], Parodi [12], Fritsche and Steinhart, [3]). It can be explained with the different formulation of the diet, the variance in the genotypes of the animals, and perhaps the alteration among the applied analytical methods. The average of the CLA content of milk fat was 1.1% that is slightly higher than Precht and Molkentin [13] determined, and the extreme values are also closer to each other. In the case of the other fatty acids present, the results are similar to the results of the other authors that are not cited in this article.

In sum it can be concluded that the majority of the saturated fatty acids dropped to a minimum value during summer and reached their maximum during winter and the early spring months. In contrast to this, the amount of unsaturated fatty acids, including CLA has the highest concentration during the summer, and the lowest concentration was measured in winter and the early spring months. The findings of the present work are in good agreement with the results of the other authors regarding to the season variation tendency. In the case of the absolute values there is a slight difference. Based on the results it can be concluded that the milk fat in summer consists of more linoleic acid, linolenic acid, oleic acid and CLA than in winter – independently of the variety of cow – and therefore the milk in summer is more suitable for the purpose of health preservation. Since the animals were kept under identical feeding conditions – mainly they consumed grass in summer and hay and silage in winter – the higher CLA content of the summer milk can be associated with the higher unsaturated fatty acid content of pasture grass, maybe the higher CLA content or the influence of the higher ultraviolet radiation.

In the future this experiment needs to be repeated with higher number of individuals and repetitions in order to achieve statistical analysis for studying the factors that could affect the fatty acid composition of the milk fat, that is feeding, variety of cows and others. In the next experiment the fatty acid composition of the forage will also be determined, because it is assumed that

the pasture grass contains more CLA-precursor than the preserved fodder, and the connection between the fatty acid composition of feed and CLA content of milk fat will be evaluated.

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