Thesis of the Ph.D. dissertation

CHANGING THE FATTY ACID COMPOSITION IN MILK FAT WITH ANIMAL NUTRITION METHODS

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1. Prelude and Objectives of Ph.D. dissertation

The objective of the dairy cow sector is to produce quality milk and dairy products. A substantial part of human needs for animal protein can be covered with milk and dairy products easily in, and in addition, according to KUKOVICS (2001) out of the foods that can be found on Earth the dairy products are the richest in bio-active materials. In our country and in many other European countries, the preserved forages (maize silage, alfalfa senage), as well as a variety of mass feed establish the basis of the feed of ruminants. As a result of the mono diet nutrition of dairy cows the fatty acid composition of the cow milk can be unfavourable for human health (ELGERSMA et al., 2006), whereas in the last decades, with regard to the health protective nutrition, a bigger emphasis was placed on producing milk and dairy products with more favourable fatty acid composition.

Lifestyle and nutrition have an important part in national economy to preserve and improve the health status of the population. The change in the consumer habit towards this direction might establish a quality production resulting in mass production, where the main objective is to produce food, including milk and dairy products, which means more than traditional food. Nowadays food products appear in human diet not only as sources of nutrients but also as multi-functional products with additional value.

Based on the available literature, the fat-sources and various preparations (full fat seeds, vegetable oils, calcium soap) used in the diet of dairy cows might influence not only the milk production but also the nutrient content of the milk and the fatty acid composition of milk fat (SCHMIDT et al., 2000; FÉBEL and VÁRHEGYI, 2007). SCHMIDT et al., (2008) claim that in case of fat-supplements that are rich in linoleic and linolenic acid the correlation coefficient of the relationship between the feedstuff and the fatty acid composition of milk (r) was 0.68. The latter result proves that the fatty acid composition of milk can be influenced by nutrition in ruminants as well. PERÉDI (2002) believes the animal products with an increased amount of n-3 fatty acid might be a possibility to solve the problem of low n-3 fatty acid consumption of Hungarian population. A number of studies considers the use of oilseeds and their by-products as perspective in the nutrition of farm animals (LIVINGSTONE et al., 2015; PETIT, 2002, 2003; MURPHY et al., 1995a,b).

Out of the oilseeds, the full fat linseed and rape seed can be such natural fat supplement, while out of the by-products it is the cold-pressed linseed. The linseed and the rapeseed contain unsaturated fatty acids in large quantities, which are important for human nutrition and have an outstanding role in the health preservation of the human body.

In addition to grazing, feeding green fodder, and applying the reasonable proportion of mass-feed and fodder, the feeding of oilseeds allows the production of milk fat with more favourable fatty acid composition, and a higher polyunsaturated fatty acid concentration and narrower n6 / n3 ratio.

The objective of the research was to find a feed supplement, which may favourably influence the fatty acid composition of milk fat, with particular regard to n-3 fatty acid content. It was also important to consider the simple usage of the chosen natural feed supplement, which is high in n-3 fatty acid, on large-scale dairy farms.

Another objective was to investigate what results can be reached concerning the alteration of the fatty acid composition in milk fat, when feeding animals with oil seeds (full fat linseed and full fat rapeseed) as well as with the by-products of linseed-oil production (cold-pressed linseed).

The influence of certain feed supplements on the quality of milk and on the milk fat content was also analysed, which parameters are extremely important in addition to the continuous production.

The following issues are to be investigated:

- In order to improve the fatty acid composition of milk fat, can the oilseeds and the by-products of the oil seed production be fitted into the nutrition of dairy cattle kept in continuous production and under intensive conditions?
- Out of the possible feed supplements available in Hungary, which natural feed supplements that are rich in n-3 fatty acid are suitable to alter the fatty acid composition of milk fat effectively?
- How and to what extent the portions of feed supplements will alter the most important production parameters (milk quality, nutritional value)?
- How will the saturated and unsaturated fatty acid composition of milk fat change after applying the feed supplements?
- What differences can be seen in the fatty acid composition of milk fat concerning the n6/n3 rate when using a certain feed supplement, and also how will this value fit in the "ideal" n6/n3 rate in human nutrition?
- Is feeding the animals with the raw, unprocessed (full fat) form of the chosen feed supplement more effective to alter the fatty acid composition of the milk fat?

- Which portions of the feed supplement (oilseeds, cold-pressed linseed) result in considerable modification in the fatty acid composition of milk fat?

The research was designed to present the benefits of applying oilseeds in animal nutrition and the possibility of the usage in practice.

2. Matherial and Methodes

2.1. The material and method of the examinations

The research was carried out according to the objectives, which means the involvement of several intensive dairy cattle farms. The Holstein Friesian cattle farms can be found in *Hajdú-Bihar County* in Hungary. The schedule on the farm was taken into account every occasion during the examinations, which was a prerequisite of the continuous production.

Hereafter the three farms will be referred to as „A", „B" and „C", where „A" is *Biharnagybajomi Dózsa Agrár Zrt., „B" is Földes Rákóczi Mezőgazdasági Kft.*-t and „C" is *Szikgát-Tej Kft.*

2.2. Planning the examinations

When planning the examinations it was a priority to select a natural raw material high in unsaturated fatty acids that can also be used simply as a feed supplement for the modification of the fatty acid composition of milk fat.

Easy availability in bulk was an important aspect as well as simple transport, storage and portioning, since it was meant for large scale usage.

Considering the research aspects and the possibilities of large scale production, out of the possible feed supplement materials, the full fat linseed high in n-3 unsaturated fatty acids as well as itsindustrial by product, the cold extruded linseed and also full fat rapeseed were selected.

The cold pressed flaxseed feed supplementation was given on farms A, B and C at the same dosage in the complete feed mix. In the experiment there were 20 Holstein Friesian multiparous cows, mostly in their second to fourth lactation, in the second lactation stage (their average milk production was 30.06 ± 2.87 kg).

When feeding full fat oil seeds, full fat rape seed and full fat linseed were used at the same dosage as part of the complete feed mix. The experiment was carried out on farms A and B with Holstein-Friesian cows. On each of the farms 12 animals participated in the study, which were multiparous cows, mostly in their second to fourth lactation, in the second lactation stage (their average milk production was 28.40 ± 4.95 kg).

The Holstein Friesian cows (A and B), which had a different dose of full fat flax fodder supplementation in the experiment (15 cows on farm A and B, respectively) were typically high yielding multiparous cows (at the start of the experiment in 185 ± 63 days of lactation), with an average milk production of 37.95 ± 7.14 kg. The fodder supplementation was part of the total feed mix on both farms.

The portions of the feed supplements are presented in *Table 1*.

Table 1. The feed supplements of the experiment

	A farm	**B farm**	**C farm**
Cold extruded linseed (kg / day / animal)	2	2	2
Full fat linseed (kg / day / animal)	1	-	-
Full fat rapeseed (kg / day / animal)	-	1	-
Full fat linseed (kg / day / animal)	1,5	1	-

The fatty acid composition and the nutritional value of the feed supplement are shown in *Table 2.* and *Table 3*.

Table 2. The fatty acid composition and the nutritional value of cold-pressed linseed

Nutritional value (%)	Cold-pressed linseed
dry matter	92,1
crude protein	32
crude fat	9,92
crude fibre	5,72
crude ash	10,9
Composition of fatty acid (%)*	
lauric acid C12:0	2,24
palmitic acid C14:0	0,05
pentadecylic acid C15:0	0,01
palmitic acid C16:0	23,64
palmitoleic acid C16:1	0,03
margaric acid C17:0	0,04
stearic acid C18:0	12,76
oleic acid C18:1n9	15,65
linoleic acid C18:2n6	10,80
arachidic acid C20:0	0,8
γ-linolenic acid C18:3n6	19,96
eikozénsav C20:1	0,16
α-linolenic acid C18:3n3	25,75
eicosenoic acid C20:2	0,04
behenic acid C22:0	0,8
eicosatrienoic acid C20:3n6	0,04
arachidonic acid C20:4n6	0,01
lignoceric acid C24:0	0,04
docosapentaenoic acid C20:5	0,05

*fatty acid methyl esters relative % by weight

Table 3: The fatty acid composition and the nutritional value of the full fat linseed and rapeseed

Nutritional value (%)	Full fat linseed	Full fat rapeseed
dry matter	93,6	92,8
crude protein	21,6	26,5
crude fat	40,4	33,5
crude fibre	5,9	5,9
crude ash	3,2	4,3
Composition of fatty acid (%)*		
lauric acid C12:0	0	0,02
palmitic acid C14:0	0,07	0,1
pentadecylic acid C15:0	0,02	0,04
palmitic acid C16:0	5,47	5,18
palmitoleic acid C16:1	0,05	0,22
margaric acid C17:0	0,08	0,06
stearic acid C18:0	3,01	1,93
oleic acid C18:1n9	18,53	57,39
linoleic acid C18:2n6	14,96	19,6
arachidic acid C20:0	0,12	0,61
γ-linolenic acid C18:3n6	0,21	0,05
eikozénsav C20:1	0,16	1,58
α-linolenic acid C18:3n3	55,98	8,06
eicosenoic acid C20:2	0,06	0,1
behenic acid C22:0	0,12	0,34
eicosatrienoic acid C20:3n6	0,04	0
erucic acid C22:1n9	0	0,83
eicosatrienoic acid C20:3n6	0,1	0
arachidonic acid C20:4n6	0,02	0,03
lignoceric acid C24:0	0,09	0,21
docosapentaenoic acid C20:5	0,07	0

* fatty acid methyl esters relative % by weight

The changes of milk quantity and milk composition were concluded from the milking trial data of *Állattenyésztési Teljesítményvizsgáló Kft* carried out every month.

While feeding the animals with cold-pressed linseed, as well as with full fat linseed and rapeseed, milk samples were taken to examine the fatty acid composition of milk fat twice during the experiment, before and at the end of feeding the animals with the feed supplement.

In case of feeding a different portion of full fat linseed, milk samples were taken three times, before feeding the feed supplement, in mid-time, and at the end of the experiment period. Sample-taking was scheduled to the milking order every time, and they were taken at the same milking period. The samples were stored in refrigerated conditions then they were taken to the Chemistry Institution of Kaposvár University, Faculty of Animal Sciences, to determine the parameters.

2.3. Applied analytic and chemical methods

The dry matter, raw protein, crude fat, crude fibre and crude ash content of solid fat, full fat rape seeds and cold pressed flax seeds, as well as that of forage fed to the animals were measured with the methods recommended in the Hungarian Code of Conduct (2004) (Hungarian Standard).

The fatty acid analysis of the milk samples was carried out by the Analytics Laboratory of the University of Kaposvár, Faculty of Animal Sciences and the Laboratory of the Centre of Agricultural Sciences MÉK Agricultural Equipment Centre.

2.4. Data processing and statistical methods

In case of the production parameters the data of the milking trials of the three periods were taken into account. Milking trials were carried out each month. The data of the amount of milk produced during the lactation and also the data of the milk composition were gathered by the programme called RISKA.

When testing the fatty acid composition of milk fat, the data of the analytical analysis of the milk samples taken during the given periods were used.

The estimation of the lactation curves and drawing the actual lactation curves was carried out according to the data of the milking trials gathered from the RISKA programme. The function for the estimation of the lactation curve was calculated with the Wood formula (WOOD, 1969) based on the work of SZŰCS et al. (1982).

SPSS for Windows 22.0 programme was used for the analysis. The data of cold-pressed linseed feeding as well as the full fat linseed and rapeseed data were analyzed by the method of independent-sample T-test. The different dose full fat linseed data were analyzed by the method of variance. Levene-test was applied to examine homogeneity. Tamhane test (in case of heterogeneity) and the LSD test (in case of homogeneity) were used to compare the group-pairs. Univariate multi-factor variance analysis (Univariate) was used when studying the interaction of the farm and the dose effect.

The results obtained during the experiment are presented in tables or in graphical form. Milking trial data and the values of the 95% confidence interval are presented in the graphs. Significant differences ($P<5\%$) are marked with different letters.

3. Results

3.1. Changes in milk yield and milk composition after applying the feed supplements

To sum up the results of the experiment it can be concluded that the cold-pressed linseed feed supplement did not have a negative influence on the milk yield. The amount of milk increased on farm A during feeding, while on farm C after feeding. On farm B the experiment did not have a notable effect on milk yield. The normal lactation curves were not affected considerably by the feed supplements. Milk fat decrease was observed on farm A, however on the other two farms the milk fat percentage was not influenced by the feed supplements. The amount of milk protein grew after feeding on farm B, while on farms A and C the change was not considerable.

When applying full fat oilseed feed supplements, the milk yield on farm A decreased after feeding. No alteration was observed in the milk yield on farm B. None of the feed supplements affected the milk fat percentage notably. The milk protein percentage fell back on farm A during feeding, while on farm B the change was not determinative. The normal lactation curves were not affected considerably by the feed supplements.

After feeding the different dose full fat linseed the milk yield decreased on both farms. The data of farm B showed that the milk fat content increased after feeding, while on farm A there was no difference. The amount of milk protein was not influenced by the feed supplement in either case.

3.2. The effect of feed supplements on the fatty acid composition of milk fat

The objective was to find out how the selected feed supplements affect the changes of saturated fatty acids and the mono- and polyunsaturated fatty acids of milk fat in case of the milking cows of a given large-scale farm. For this purpose, the fatty acid composition of the milk fat of cows in the experiment was examined in the period before and after the research, and also in case of feeding a different dose full fat linseed in three periods, before, after and during the experiment.

3.2.1. The effect of cold-pressed linseed feed supplement on the fatty acid composition of milk fat

Table 4: The effect of cold-pressed linseed on the monounsaturated fatty acids of milk fat (Farm A)

Fatty acids (%)*	Before the experiment	After the experiment	P
myristoleic acid	0,79 ± 0,12	0,89 ± 0,17	ns
palmitoleic acid	1,28 ± 0,13	1,25 ± 0,19	ns
elaidic acid	2,88 ± 0,16	3,32 ± 0,12	<5%
oleic acid	24,45 ± 1,08	26,40 ± 0,62	<5%
eicosenoic acid	0,05 ± 0,01	0,05 ± 0,00	ns
ΣMUFA	29,44 ± 10,43	31,92 ± 11,26	<5%

* fatty acid methyl esters relative % by weight

When considering the results of the monounsaturated fatty acids (*Table 4*) the following can be concluded. The increase of the proportion of elaidic acid and oleic acid in the experiment period was determinative compared to the values of the period before the examinations. The total proportion of monounsaturated fatty acids shifted to a positive direction when the two experiment periods were compared.

The results concerning the polyunsaturated fatty acid composition are shown in *Table 5*, where the data of the fatty acid composition before feeding the supplements are compared with that of after the experiment.

Table 5: The effect of cold-pressed linseed on the polyunsaturated fatty acids of milk fat (Farm A)

Fatty acids (%)*	Before the experiment	After the experiment	P
linoleic acid	3,15 ± 0,17	3,50 ± 0,17	<5%
conjugated linoleic acid	0,53 ± 0,03	0,71 ± 0,06	<5%
γ-linolenic acid	0,5 ± 0,02	0,02 ± 0,01	ns
α-linolenic acid	0,37 ± 0,04	0,51 ± 0,03	<5%
eicosadienoic acid	0,03 ± 0,01	0,03 ± 0,01	ns
eicosatrienoic acid	0,21 ± 0,02	0,16 ± 0,02	<5%
arachidonic acid	0,26 ± 0,02	0,23 ± 0,02	<5%
eicosapentaenoic acid	0,03 ± 0,00	0,04 ± 0,00	ns
docosapentaenoic acid	0,08 ± 0,01	0,07 ± 0,01	ns
ΣPUFA	4,72 ± 1,00	5,27 ± 1,12	<5%

* fatty acid methyl esters relative % by weight

In case of most polyunsaturated fatty acids there was a positive change after the experiment. The most significant change, a more than one and a half times increase, was observed in the proportion of α-linolenic acid and a conjugated linoleic acid after the experiment period. The concentration of linoleic acid also increased. The increase of α-linolenic acid is considered determinative as for narrowing the n6/n3 fatty acid proportion of milk fat, and the conjugated linoleic acid as a bioactive substance, has numerous favourable characteristics in human nutrition. As for the arachidonic acid, a smaller decrease was observed. The total rate of the polyunsaturated fatty acids in milk fat showed an increase when compared to the data of the period before the experiment.

Table 6 presents the monounsaturated fatty acid composition of the milk samples from farm B when applying cold-pressed linseed feed supplement and the period before the experiment.

Table 6: The effect of cold-pressed linseed on the monounsaturated fatty acids of milk fat (Farm B)

Fatty acids (%)*	Before the experiment	After the experiment	P
myristoleic acid	0,83 ± 0,09	1,00 ± 0,11	<5%
palmitoleic acid	1,47 ± 0,13	1,44 ± 0,16	ns
elaidic acid	1,68 ± 0,16	4,24 ± 1,29	<5%
oleic acid	22,11 ± 1,24	21,74 ± 1,20	ns
eicosenoic acid	0,06 ± 0,01	0,05 ± 0,01	ns
ΣMUFA	32,63 ± 9,00	28,48 ± 9,11	ns

* fatty acid methyl esters relative % by weight

When feeding cold-pressed linseed under the conditions of the given large-scale farm, no considerable result was achieved in case of the majority of fatty acids belonging to the MUFA group. The intensive increase (more than 2.5 times) in the proportion of elaidic acid and the positive change in the concentration of myristoleic acid was conspicuous, though. The change in the total proportion of monounsaturated fatty acids of milk fat was not significant. The data of the polyunsaturated fatty acids before and after feeding cold-pressed linseed are presented in *Table 7*.

Table 7: The effect of cold-pressed linseed on the polyunsaturated fatty acids of milk fat (Farm B)

Fatty acids (%)*	Before the experiment	After the experiment	P
linoleic acid	3,03 ± 0,24	3,49 ± 0,38	ns
conjugated linoleic acid	0,31 ± 0,05	0,67 ± 0,12	<5%
γ-linolenic acid	0,06 ± 0,02	0,03 ± 0,01	ns
α-linolenic acid	0,29 ± 0,03	0,58 ± 0,08	<5%
eicosadienoic acid	0,04 ± 0,02	0,03 ± 0,00	ns
eicosatrienoic acid	0,20 ± 0,03	0,17 ± 0,01	ns
arachidonic acid	0,27 ± 0,03	0,23 ± 0,01	ns
eicosapentaenoic acid	0,03 ± 0,01	0,02 ± 0,01	ns
docosapentaenoic acid	0,07 ± 0,02	0,07 ± 0,01	ns
ΣPUFA	4,30 ± 0,96	5,30 ± 1,11	<5%

* fatty acid methyl esters relative % by weight

In case of polyunsaturated fatty acids there was an increase in conjugated linoleic acid és α-linolenic acid. In the concentration of the other polyunsaturated fatty acids no significant change was found. However, the total rate of the polyunsaturated fatty acids of milk fat was higher in the post-experiment period than before.

Table 8 presents the changes of the monounsaturated fatty acid composition after feeding cold-pressed linseed as feed supplement on farm C.

Table 8: The effect of cold-pressed linseed on the monounsaturated fatty acids of milk fat (Farm C)

Fatty acids (%)*	Before the experiment	After the experiment	P
myristoleic acid	0,80 ± 0,13	0,69 ± 0,07	ns
palmitoleic acid	1,27 ± 0,14	1,14 ± 0,12	ns
elaidic acid	2,43 ± 0,13	2,86 ± 0,20	<5%
oleic acid	25,37 ± 1,60	25,84 ± 1,23	ns
eicosenoic acid	0,07 ± 0,00	0,06 ± 0,00	ns
ΣMUFA	29,94 ± 10,87	30,59 ± 11,07	ns

* fatty acid methyl esters relative % by weight

Regarding the changes of monounsaturated fatty acids it can be concluded that apart from the increased proportion of elaidic acid in milk fat, there was no positive change in monounsaturated fatty acids and in their total proportion after applying cold-pressed feed supplements.

Table 9 details the polyunsaturated fatty acid composition of the milk samples from farm C in the period after the experiment applying cold-pressed linseed as feed supplement and in the period without feeding the supplement.

Table 9: The effect of cold-pressed linseed on the monounsaturated fatty acids of milk fat (Farm C)

Fatty acids (%)*	Before the experiment	After the experiment	P
linoleic acid	2,62 ± 0,25	2,81 ± 0,07	ns
conjugated linoleic acid	0,46 ± 0,05	0,57 ± 0,05	ns
γ-linolenic acid	0,03 ± 0,01	0,02 ± 0,01	ns
α-linolenic acid	0,31 ± 0,02	0,42 ± 0,11	ns
eicosadienoic acid	0,04 ± 0,02	0,03 ± 0,00	ns
eicosatrienoic acid	0,14 ± 0,01	0,15 ± 0,01	ns
arachidonic acid	0,24 ± 0,02	0,23 ± 0,01	ns
eicosapentaenoic acid	0,03 ± 0,01	0,03 ± 0,01	ns
docosapentaenoic acid	0,07 ± 0,02	0,06 ± 0,01	ns
ΣPUFA	3,93 ± 0,83	4,32 ± 0,89	<5%

* fatty acid methyl esters relative % by weight

When analysing the rate of the polyunsaturated fatty acids it is obvious that none of the fatty acids produced any remarkable changes. At the same time the proportion of polyunsaturated fatty acids in milk fat increased and it proved to be significant.

The cold-pressed linseed feed supplement resulted in a decreased concentration of palmitic acid on all three farms. Further decrease was on farm A, considering the ratio of pentadecylic acid and margaric acid and the total ratio of the saturated fatty acids in milk fat also decreased. The results of farm B showed an increase in the ratio of several fatty acids (undecylic acid, tridecylic acid, pentadecylic acid). On farm C the concentration of tridecylic acid, pentadecylic acid and margaric acid fell back after feeding. The analysis of monounsaturated fatty acids showed the increase in the proportion of elaidic acid in case of the three farms. Furthermore, the concentration of oil acid on farm A, while the ratio of myristoleic acid on farm B also increased. Out of the polyunsaturated fatty acids, there was an increase of linoleic acid and α-linolenic acid after feeding on both farms A and B, and the results on farm A also justified the increase in the concentration of linoleic acid. On farm C there was no significant change in the proportion of the examined polysaturated fatty acids. The proportion of the n6/n3 fatty acids was not influenced by the feed supplements on any of the farms.

Considering the observations and proposals of the relevant professional literature as well as the aspects of large scale production it was expedient to apply a by-product of linseed oil production as feed supplement for milking cows.

3.2.2. The effect of full fat oilseed feet supplements on the fatty acid composition of milk fat

The changes in the concentration of monounsaturated fatty acids in case of feeding full fat linseed feed supplements are shown in *Table 10.*

Table 10: The effect of full fat linseed on the monounsaturated fatty acids of milk fat (Farm A)

Fatty acids (%)*	Before the experiment	After the experiment	P
myristoleic acid	0,90 ± 0,21	1,60 ± 0,24	ns
palmitoleic acid	1,71 ± 0,17	1,26 ± 0,19	ns
elaidic acid	2,19 ± 0,42	1,38 ± 0,46	<5%
oleic acid	22,65 ± 1,17	25,81 ± 1,61	<5%
eicosenoic acid	0,05 ± 0,004	0,07 ± 0,02	ns
ΣMUFA	27, 51 ± 5,50	29,70 ± 5,94	ns

* fatty acid methyl esters relative % by weight

In case of the two modifications of C18:1 isomer – elaidic acid and oleic acid – a significant change was observed in the post-experiment period. At the same time the total rate of the monounsaturated fatty acids in milk fat did not change considerably when comparing the two examined periods.

The changes in the proportion of the polyunsaturated fatty acids before and after feeding full fat linseed feed supplements are presented in *Table 11*.

Table 11: The effect of full fat linseed on the polyunsaturated fatty acids of milk fat (Farm A)

Fatty acids (%)*	Before the experiment	After the experiment	P
linoleic acid	2,78 ± 0,24	2,57 ± 0,19	ns
conjugated linoleic acid	0,52 ± 0,04	0,62 ± 0,05	<5%
γ-linolenic acid	0,02 ± 0,01	0,06 ± 0,07	ns
α-linolenic acid	0,27 ± 0,03	0,60 ± 0,05	<5%
eicosadienoic acid	0,03 ± 0,01	0,04 ± 0,01	<5%
eicosatrienoic acid	0,14 ± 0,02	0,14 ± 0,05	ns
arachidonic acid	0,17 ± 0,02	0,18 ± 0,03	ns
docosapentaenoic acid	0,01 ± 0,00	0,05 ± 0,03	<5%
ΣPUFA	3,93 ± 0,94	4,27 ± 0,85	ns

* fatty acid methyl esters relative % by weight

When studying the changes of the proportion of the polyunsaturated fatty acids it was found that there was a statistically proved increase in case of most fatty acids. In the proportion of a conjugated linoleic acid and a α-linolenic acid the increase was considerable in the post-experiment period. Further differences were observed in the concentration of the eicosadienoic acid and a docosapentaenoic acid after feeding the supplements, and it is particularly important regarding the endeavour to reach the optimal n6/n3 ratio.

The following observations were made concerning the monounsaturated fatty acid composition of milk fat on farm B, in case of feeding full fat rapeseed as feed supplement (*12. Table*).

Table 12: The effect of full fat rapeseed on the monounsaturated fatty acids of milk fat (Farm B)

Fatty acids (%)*	Before the experiment	After the experiment	P
myristoleic acid	0,90 ± 0,13	0,98 ± 0,13	ns
palmitoleic acid	1,66 ± 0,20	1,80 ± 0,39	ns
elaidic acid	1,53 ± 0,26	1,40 ± 0,32	ns
oleic acid	18,17 ± 1,06	19,56 ± 1,05	<5%
eicosenoic acid	0,05 ± 0,004	0,07 ± 0,02	ns
ΣMUFA	22,31 ± 7,69	23,88 ± 8,39	<5%

* fatty acid methyl esters relative % by weight

Out of the monounsaturated fatty acids, there was an increase in case of the proportion of an oleic acid. The total rate of the monounsaturated fatty acids in milk fat was significantly higher. The results of the polyunsaturated fatty acid composition of milk fat can be seen in the overview of *Table 13*, in the period before and after applying full fat rapeseed as feed supplement.

Table 13: The effect of full fat rapeseed on the polyunsaturated fatty acids of milk fat (Farm B)

Fatty acids (%)*	Before the experiment	After the experiment	P
linoleic acid	2,57 ± 1,11	2,60 ± 0,21	ns
conjugated linoleic acid	0,33 ± 0,01	0,49 ± 0,12	ns
γ-linolenic acid	0,03 ± 0,01	0,05 ± 0,04	ns
α-linolenic acid	0,39 ± 0,01	0,45 ± 0,09	ns
eicosadienoic acid	0,05 ± 0,01	0,04 ± 0,02	ns
eicosatrienoic acid	0,16 ± 0,02	0,15 ± 0,02	ns
arachidonic acid	0,25 ± 0,03	0,24 ± 0,03	ns
docosapentaenoic acid	0,07 ± 0,01	0,06 ± 0,03	ns
ΣPUFA	3,85 ± 0,85	3,95 ± 0,86	ns

* fatty acid methyl esters relative % by weight

There was no positive alteration neither in the proportion of the polyunsaturated fatty acids, nor in the total rate of the polyunsaturated fatty acids when applying full fat rapeseed feed supplement.

When analysing the saturated fatty acids after feeding the full fat oil-seed feed supplement a decrease was found in a greater number of fatty acid ratio. On both farms the concentration of caprylic acid, a capric acid, a lauric acid és a myristic acid decreased and the total ratio of saturated fatty acids of milk fat also reduced after the feeding. Furthermore, on farm A the ratio of palmitic acid and margaric acid fell back as well, however the concentration of stearic acid és a behenic acid increased after feeding the feed-supplement. The analysis of farm B showed further decrease in the proportion of undecylic acid and stearic acid as well. The analysis of monounsaturated fatty acids indicated an increase in the proportion of oleic acid on both farms after feeding the feed supplement, at the same time on farm B the concentration of elaidic acid was lower. The analysis of the polyunsaturated fatty acids of the results of farm A found an increase in the proportion of the conjugated linoleic acid , az α-linolenic acid, az eicosadienoic acid and docosapentaenoic acid, while on farm B no relevant change can be observed after using the feed supplements. None of the farms had any determinative change in the n6/n3 fatty acid ratio after the experiment.

3.2.3. The effect of different dose full fat linseed on the monounsaturated fatty acid composition of milk fat

The changes in the composition of monounsaturated fatty acids in the three experiment period when applying higher doses of full fat linseed feed supplements are presented in *Table 14*.

Table 14: The effect of full fat linseed on the monounsaturated fatty acids of milk fat

Fatty acids (%)*	Before the experiment	During the experiment	After the experiment	P
myristoleic acid	$0,73^{a} \pm 0,15$	$0,82 \pm 0,15$	$0,99^{b} \pm 0,22$	<5%
palmitoleic acid	$1,40 \pm 0,32$	$1,51 \pm 0,25$	$1,57 \pm 0,36$	ns
heptadecanyl acid	$0,18 \pm 0,06$	$0,21 \pm 0,06$	$0,21 \pm 0,06$	ns
elaidic acid	$1,41 \pm 0,48$	$1,60 \pm 0,41$	$1,28 \pm 0,35$	ns
oleic acid	$20,77^{a} \pm 2,76$	$27,08^{b} \pm 3,37$	$25,50^{b} \pm 2,91$	<5%
eicosenoic acid	$0,04^{a} \pm 0,01$	$0,05^{b} \pm 0,01$	$0,05 \pm 0,01$	<5%
ΣMUFA	$24,53^{a} \pm 8,19$	$31,28^{b} \pm 10,73$	$29,61^{b} \pm 10,13$	<5%

* fatty acid methyl esters relative % by weight
The averages marked with letters in the horizontal lines are significantly different from each other, P<5%

When studying the changes of the monounsaturated fatty acids there was an increasing tendency in the proportion of myristoleic acid when comparing the various experiment periods. In case of an oleic acid a strong increase was observed between the pre-experiment period and the following experiment period, which fell back somewhat by the end of the examinations, while in case of eicosenoic acid the concentration growth was observed when comparing the pre-experiment and the middle periods. The rate of monounsaturated fatty acids in milk fat showed significant growth in the middle period of the experiment, however its reduction at the end of the examinations was not remarkable.

As for the polyunsaturated fatty acids of milk fat (*Table 15*), in case of most fatty acids statistically proven changes were achieved with the increased doses of full fat linseed feed supplements in the various periods of the feeding.

Table 15: The effect of full fat linseed on the polyunsaturated fatty acids of milk fat

Fatty acids (%)*	Before the experiment	During the experiment	After the experiment	P
linoleic acid	2,70 ± 0,49	2,56 ± 0,43	2,43 ± 0,35	ns
conjugated linoleic acid	0,30^{a} ± 0,04	0,46^{b} ± 0,07	0,38^{c} ± 0,06	<5%
γ-linolenic acid	0,04^{a} ± 0,01	0,04^{a} ± 0,01	0,06^{b} ± 0,01	<5%
α-linolenic acid	0,34^{a} ± 0,05	0,60^{b} ± 0,01	0,42^{c} ± 0,06	<5%
eicosadienoic acid	0,02 ± 0,005	0,02 ± 0,01	0,02 ± 0,007	ns
eicosatrienoic acid	0,14^{a} ± 0,04	0,08^{b} ± 0,02	0,10 ± 0,05	<5%
arachidonic acid	0,20^{a} ± 0,04	0,16^{b} ± 0,02	0,16^{b} ± 0,02	<5%
eicosapentaenoic acid	0,03^{a} ± 0,07	0,04^{b} ± 0,008	0,04^{b} ± 0,008	<5%
eicosapentaenoic acid	0,07^{a} ± 0,01	0,07 ± 0,01	0,08*b* ± 0,008	<5%
ΣPUFA	3,82^{a} ± 1,35	4,02^{b} ± 1,37	3,65 ± 1,26	<5%

* fatty acid methyl esters relative % by weight
The averages marked with letters in the horizontal lines are significantly different from each other, P<5%

When analysing the polyunsaturated fatty acids the increase of the proportion of conjugated linoleic acid was observed in the pre-experiment and the middle period of the experiment, however it was lower at the end of the examinations. The increase in the concentration of the γ-linolenic acid was pronounced before and after the experiment period. The increase in the rate of α-linolenic acid was significant in the pre-experiment period and also in the middle of the experiment, however it was lower by the end of the examinations. The concentration of eicosatrienoic acid decreased a little in the pre-experiment and in the middle period. The proportion of arachidonic acid was also lower by the end of the experiment, at the same time there was a small, statistically proven increase in the proportion of eicosapentaenoic acid and a eicosapentaenoic acid when comparing the pre-experiment and the experiment periods. Considerable change in the total proportion of polyunsaturated fatty acids was achieved in the middle period of the experiment.

Table 16 presents the fatty acid composition of monounsaturated fatty acids on farm B in case of applying the normal dose full fat linseed feed supplements.

Table 16: The effect of full fat linseed on the monounsaturated fatty acids of milk fat

Fatty acids (%)*	Before the experiment	During the experiment	After the experiment	P
myristoleic acid	0,85 ± 0,27	0,88 ± 0,24	0,97 ± 0,33	ns
palmitoleic acid	1,78 ± 0,37	1,52 ± 0,38	1,82 ± 0,55	ns
heptadecanyl acid	$0{,}20^{a}$ ± 0,05	$0{,}16^{b}$ ± 0,02	$0{,}15^{b}$ ± 0,03	<5%
elaidic acid	$2{,}39^{a}$ ± 0,96	$1{,}57^{b}$ ± 0,23	$1{,}40^{b}$ ± 0,28	<5%
oleic acid	$21{,}33^{a}$ ± 3,36	$24{,}71^{b}$ ± 2,96	22,51 ± 2,88	<5%
eicosenoic acid	$0{,}05^{a}$ ± 0,01	$0{,}05^{a}$ ± 0,01	$0{,}04^{b}$ ± 0,004	<5%
ΣMUFA	26,60 ± 8,33	28,89 ± 9,77	26,75 ± 8,76	ns

* fatty acid methyl esters relative % by weight

The averages marked with letters in the horizontal lines are significantly different from each other, P<5%

In case of most monounsaturated fatty acids the changes were statistically proven. When comparing the pre-experiment period and the experiment periods a decrease in the proportion of heptadecanyl acid, elaidic acid and an eicosenoic acid was observed. In case of the oleic acid the increase was considerable when comparing the pre-experiment and the middle experiment period. During the examinations no proven change was observed in the proportion of the monounsaturated fatty acids.

Studying the changes of the polyunsaturated fatty acids in case of applying full fat linseed feed supplements, the increase in the proportion of several fatty acids were proven (*Table 17.*).

Table 17: The effect of full fat linseed on the polyunsaturated fatty acids of milk fat

Fatty acids (%)*	Before the experiment	Middle the experiment	After the experiment	P
linoleic acid	$3{,}19^{a}$ ± 0,28	$2{,}36^{b}$ ± 0,25	$2{,}28^{b}$ ± 0,22	<5%
conjugated linoleic acid	0,45 ± 0,08	0,48 ± 0,06	0,45 ± 0,11	ns
γ-linolenic acid	$0{,}4^{a}$ ± 0,01	$0{,}03^{b}$ ± 0,006	$0{,}03^{b}$ ± 0,006	<5%
α-linolenic acid	$0{,}23^{a}$ ± 0,02	$0{,}58^{b}$ ± 0,07	$0{,}59^{b}$ ± 0,11	<5%
eicosadienoic acid	0,02 ± 0,01	0,02 ± 0,01	0,03 ± 0,04	ns
eicosatrienoic acid	$0{,}13^{a}$ ± 0,03	$0{,}08^{b}$ ± 0,02	$0{,}07^{b}$ ± 0,02	<5%
Arachidonic acid	$0{,}20^{a}$ ± 0,03	$0{,}14^{b}$ ± 0,02	$0{,}13^{b}$ ± 0,02	<5%
eicosapentaenoic acid	$0{,}01^{a}$ ± 0,005	$0{,}03^{b}$ ± 0,01	$0{,}03^{b}$ ± 0,01	<5%
docosapentaenoic acid	$0{,}05^{a}$ ± 0,01	$0{,}07^{b}$ ± 0,01	0,06 ± 0,01	<5%
ΣPUFA	$4{,}33^{a}$ ± 1,56	$3{,}79^{b}$ ± 1,28	$3{,}69^{b}$ ± 1,25	<5%

* fatty acid methyl esters relative % by weight

The averages marked with letters in the horizontal lines are significantly different from each other, P<5%

When studying the polyunsaturated fatty acids it can be concluded that the decrease in the proportion of linolsav és a γ-linolénsav in the comparison of the pr-experiment and middle experiment periods was statistically proven. The increase of the proportion of α-linolenic acid was outstanding when the experiment periods were compared. The statistically proven decrease of eicosatrienoic acid, the small, however significant increase of a docosapentaenoic acid was observed. The total proportion of polyunsaturated fatty acids in milk fat indicated a reducing tendency in the comparison of the experiment periods.

Applying increased and normal doses of full fat linseed feed supplements, the concentration of lauric acid, a palmitic acid and a behenic acid decreased as well as the total rate of the saturated fatty acids of the milk fat, according to the results of farm A, however there was an increase in the rate of trydecylic acid. After the feeding, a decreasing tendency can be observed on farm B in the proportion of lauric acid, a trydecylic acid, a pentadecylic acid, a margaric acid, an arachidic acid, a heneicosylic acid and a behenic acid. At the same time the rate of palmitic acid and a stearic acid was higher on farm B. Out of the monounsaturated fatty acids, the proportion of oleic acid was higher on both farms. In the results of farm A, further growth can be observed in the concentration of eicosenoic acid and also in the total rate of monounsaturated fatty acids, while on farm B the proportion of eicosenoic acid, a elaidic acid decreased. Out of the polyunsaturated fatty acids of the milk fat there was an increase in the proportion of α-linolenic acid, an eicosapentaenoic acid and a docosapentaenoic acid on both farms, after applying the feed supplements. On farm A further positive changes were observed in the concentration of conjugated linoleic acid and a γ-linolenic acid, while the proportion of the two fatty acids (eicosatrienoic acid, arachidonic acid) decreased. On farm B there was a decrease in the proportion of linoleic acid, a γ-linolenic acid, an eicosatrienoic acid and an arachidonic acid after the feeding. On farm A the total rate of the polysaturated fatty acids increased during the feeding, while it decreased on farm B after the feeding. The n6/n3 fatty acid ratio was not influenced by the feed supplements on either farm.

4. New scientific results

1. Full fat linseed and full fat rape seed that are rich in n-3 fatty acids given to the animals daily and per 1 kg of feed per animal, did not reduce the fat content of milk

2. Out of the examined unsaturated fatty acid sources (cold pressed flax seed, full fat flax seed, full fat rape seed) it was the whole fat rape seeds (1 kg / day / cow) that did not have a significant effect on the polyunsaturated fatty acid composition of milk fat.

3. In addition to the appropriate feeding processes in accordance with Hungarian practice, the full fat linseed, as a natural n-3 fatty acid source, increased the proportion of α-linolenic acid, eicosapentaenoic acid and docosapentaenoic acid, which are significant from human-health point of view.

4. The effect of cold-pressed flax seed was more pronounced in the overall ratio of polyunsaturated fatty acids than that of full fat linseed and full fat rapeseed

5. In addition to providing preserved fodder for Hungarian dairy herds, the supplementation of cold pressed flax seed (2 kg / day / cow), full fat linseed (1-1.5 kg / day / cow), and full fat rape seed (1 kg / day / cow) did not change the ratio of n-6 / n-3 fatty acids in milk fat.

6. The decrease in the ratio of palmitic acid, which proved to be related to cardiovascular diseases, was significant following the feeding of both cold pressed flaxseed and full fat linseed.

5. Practical usability of the results

1. The research has shown that cold-pressed flaxseed and full-fat oilseeds can be integrated into the feeding practice of intensively kept Holstein-Friesian cattle producing under industrial conditions in Hungary, without adversely affecting large-scale production technology.

2. Our results have proved that in addition to providing mass feed, which is characteristic in Hungarian dairy production, the supplementary full fat linseed as a natural n-3 fatty acid source increased the proportion of α-linolenic acid, eicosapentaenoic acid and docosapentaenoic acid in milk fat, which are significant from human health point of view.

3. Based on our examination it was found that the unsaturated fatty acids (cold pressed flaxseed, full fat flax seed, full fat rape seed) in the experiment were suitable to reduce the proportion of palmitic acid in milk fat, which proved to be in connection with cardiac and vascular diseases.

4. The experiment results showed, that out of the unsaturated fatty acids (cold pressed flaxseed, full fat flax seed, full fat rape seed) in the experiment, the full fat rape seed supplementation (1 kg/day/cow) did not affect significantly the polyunsaturated fatty acid composition of milk fat.

6. Bibliography

1. ELGERSMA, A. - TAMMINGA S. - ELLEN, G. (2006): Modifying milk composition through forage. Animal Feed Science Technology 131. 3-4. 207-225
2. FÉBEL, H. – VÁRHEGYI, I. (2007): A tejzsír zsírsav-összetételének módosítása: lehetőségek, korlátok, táplálkozás-élettani szempontok. In: 326. Tudományos Kollokvium. Az MTA Élelmiszertudományi Komplex Bizottsága a Központi Élelmiszer-tudományi Kutatóintézet és a Magyar Élelmezésipari Tudományos Egyesület közös rendezésében. 2007. április 27. Budapest
3. KUKOVICS, S. (2001): A tej szerepe az emberi táplálkozásban. Új Diéta. 34.11.2-3
4. LIVINGSTONE, K.M. – HUMPHRIES, D.J. – KLIEM, K.E. – GIVENS, D.I. – REYNOLDS, C.K. (2015): Effects of forage type and extruded linseed supplementation on methane production and milk fatty acid composition of lactating dairy cows. Journal of Dairy Science. 98. 6. 4000-4011
5. MURPHY, J.J. – CONOLLY, J.F. – MCNEIL, G.P. (1995a): Effects on milk fat composition and cow performance of feeding concentrates containing full fat rapeseed and maize distillers grains on grass-silage based diets. Livestock Production Science. 44. 1. 1-11
6. MURPHY, J.J. – CONOLLY, J.F. – MCNEIL, G.P. (1995b): Effects of performance and milk fat composition of feeding full fat soyabeans and rapeseeds to dairy cows at pasture. Livestock Production Science. 44. 1. 13-25
7. PERÉDI, J. (2002): A hazai lakosság alacsony n-3 zsírsavelátottságának javítási lehetőségei. Olaj, Szappan, Kozmetika. 51. 2. 45-49
8. PETIT, H.V. (2002): Digestion, milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. Journal of Dairy Science. 85. 6. 1482-1490.
9. PETIT, H.V. (2003): Digestion, milk production, milk composition, and blood composition of dairy cows fed formaldehyde treated flaxseed or sunflower seed. Journal of Dairy Science. 86. 8. 2637-2646
10. SCHMIDT, J. – SIPŐCZ, P. – SIPŐCZ, J. (2000): Védett zsír hatása a bendőfermentációra és felhasználása a nagy tejtermelésű tehenek takarmányozásában. Állattenyésztés és Takarmányozás. 2. 49. 139-154
11. SCHMIDT, J. – PERÉDI, J. – TÓTH, T. – ZSÉDELY, E. (2008): A takarmányozás hatása az állati eredetű élelmiszerek összetételére és minőségére. In: A jövő élelmiszerei és az egészség. Szerk. NAGY, J., Debrecen. 197

12. SZŰCS, E. – MÓZSI, Z. – SZÖLLŐSI, I. – ÁCS, I. (1982): A laktációs görbe illesztése Wood-függvénnyel és a matematikai modell felhasználása a fejőstehenek tejtermelésének becslésében. Állattenyésztés és Takarmányozás. 31. 2. 115-122
13. WOOD, P.D.P. (1969): Factors affecting the shape of the lactation curve in cattle. Animal Science. 11.3. 307-316

7. List of publications related to the dissertation

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List of publications related to the dissertation

Hungarian book chapters (1)

1. **Süli, Á.**: Erjedést segítő anyagok és erjedésdinamika I.
   In: 50 év a mezőgazdaság szolgálatában Tanulmánykötet Professzor Szűcsné Dr. Péter Judit 70. születésnapja tiszteletére. Szerk.: Bodnár Károly és Horváth József, SZTE MGK, Hódmezővásárhely, 24-31, 2016. ISBN: 9789633064894

Hungarian scientific articles in Hungarian journals (5)

2. **Süli, Á.**, Béri, B., Csapó, J.: A takarmányozás hatása az állati termékekre, különös tekintettel a zsírsav-összetételére.
   Állatteny. Takarm. 64 (3), 164-177, 2015. ISSN: 0230-1814.
3. **Süli, Á.**, Béri, B., Csapó, J., Vargáné Visi, É., Borbélyné Varga, M.: Az olajos magvak felhasználásának lehetősége a tejelő tehenek takarmányozásában.
   Agrártud. Közl. 57, 67-74, 2014. ISSN: 1587-1282.
4. **Süli, Á.**, Béri, B., Csapó, J., Vargáné Visi, É.: A lenmag etetés hatása a tej zsírsavösszetételére.
   Agrártud. Közl. 51, 45-50, 2013. ISSN: 1587-1282.
5. **Süli, Á.**, Béri, B.: Funkcionális élelmiszer-előállítás lehetősége szarvasmarhával.
   Economica. 2 (1), 58-64, 2009. ISSN: 1585-6216.
6. **Süli, Á.**, Béri, B.: Hidegen sajtolt lenmag etetése szarvasmarhával.
   Agrár- Vidékfejleszt. Szle. 4 (2), 157-162, 2009. ISSN: 1788-5345.

Foreign language scientific articles in Hungarian journals (1)

7. **Süli, Á.**, Garipoglu, A. V., Csapó, J., Béri, B., Vargáné Visi, É., Jónás, E.: Dietary manipulation to increase the concentration of n-3 fatty acids in milk fat.
   Appl. Ecol. Environ. Res. 16 (2), 1553-1562, 2018. ISSN: 1589-1623.
   DOI: http://dx.doi.org/10.15666/aeer/1602_15531562
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Foreign language scientific articles in international journals (3)

8. Béri, B., Vargáné Visi, É., Lóki, K., Csapóné Kiss, Z., **Süli, Á.**, Salamon, R. V., Csapó, J.: Analysis of the fatty acid pattern of milk from current and rare cattle breeds. *Acta Univ. Sapientiae, Alim. 4*, 28-43, 2011. ISSN: 1844-7449.
9. Csapó, J., Lóki, K., Béri, B., **Süli, Á.**, Vargáné Visi, É., Albert, C., Csapóné Kiss, Z.: Colostrum and milk of current and rare cattle breeds: protein content and amino acid composition. *Acta Univ. Sapientiae, Alim. 4*, 18-27, 2011. ISSN: 1844-7449.
10. Vargáné Visi, É., **Süli, Á.**, Béri, B., Csapóné Kiss, Z., Lóki, K., Salamon, R. V., Csapó, J.: Colostrum of current and rare cattle breeds: fatty acid pattern. *Acta Univ. Sapientiae, Alim. 4*, 5-17, 2011. ISSN: 1844-7449.

Hungarian conference proceedings (1)

11. **Süli, Á.**, Béri, B.: A takarmányozás hatása a tej zsírsavösszetételére. *Anim. Welf. Etol. Tartástechnol. 5* (4), 255-262, 2009. ISSN: 1786-8440.

Foreign language conference proceedings (2)

12. **Süli, Á.**, Béri, B., Csapó, J., Berecz, E.: Oilseeds as a perspective in the nutrition on dairy farm. *Lucr. Ştiint. Zootech. Biotehnol. 50* (2), 29-33, 2017. ISSN: 1841-9364.
13. Csapó, J., Béri, B., **Süli, Á.**, Vargáné Visi, É.: Colostrum and milk of different cattle breeds as amino acid source. *Acta Agric. Slov. 100* (Suppl.), 327-331, 2012. ISSN: 1581-9175.

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