## Thesis of doctoral (Ph.D.) dissertation

## POSSIBILITY OF DEVELOPING A FUNCTIONAL FEED FOR PIGS

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#### 1. RESEARCH PLAN, OBJECTIVES

Edible fats play an important role in the energy supply of mankind since tge concentrated energy they contain is about two and a half times as much as that of contained in other food components and human organism can take up the vital essential fatty acids, the linolic acid, the arachidonic acid found only in fats of animal origin and the semi-essential linolenic acid from these fats.

In Hungary, there are no problems as regards the energy supply of the population as our foods contain adequate amounts of both fat and carbohydrate. What is more, one of the major defects in nutrition here is that too much energy taken in together with fat, is consumed at the expense of carbohydrates and that the organism does not get the essential amino acids and proteins that are vital for it in addition to energy. Considering the beneficial physiological effects of unsaturated fatty acids dietary specialists recommend consuming more less fat of animal origin and more fat of plant origin. The omega-3 fatty acid contents of our foods, however, are much higher and their omega-6 fatty acid contents are much lower than would be optimal from the point of view of nutrition.

Another reason against the consumption of animal fats is the high amount of cholesterol contained in foods of animal origin, which was earlier connected to the emergence of cardio-vascular complaints, namely that of arteriosclerosis. Now we know that there are no direct links between the cholesterol contents of blood, the cholesterol content of blood sera and the occurrence of arteriosclerosis. Despite, a lot of people have banished such excellent edible fats as e.g. butter from their diets. The extremely beneficial effects of conjugated linoleic acids (CLA) have been found out only recently and these are thought to have health protecting, anti-oxidant and anti-cancer effects. CLAs are formed in the courses of different biochemical processes in the rumens of ruminants so the meat and fat of these animals can serve as considerable sources of CLAs for humans. CLAs are not produced in the organisms of monogastric animals in a way they are produced in ruminants and so their meat contains CLAs only in traces, which CLAs get into their organisms together with their feed.

It is well known that with appropriate feeding the content of the animal, like meat/fat proportion, acid conetent can be influenced. Thus it becomes possible to produce better food and raw material for human nutrition. To realise these aims we can use the functional feeding, when we produce an animal-endproduct with the option of feeding possibilities, which represents extra value for humans from the point of nutrition. These types of food can be functional food. In Japan this type of functional food is called 'defined health usefulness

food', which are certain processed types of food that are not only nutritional but also increase certain body functions: strenghtens the immunology of the body, contribute to the prevention of illnesses like high blood pressure or diabetes, also they accelerate the recovery after an illness, they imporve the physical state and slw down the ageing (BÍRÓ et al., 1997). Accoring to European Commission Concerted Action on Functional Food Science (FUFOSE-Group) functional food is the following: Food can be called functional when beyond the appropriate nutritional effects it has positive effects on one or more aim-function, thus an improved health condition or a better general state of health and/or the decrease of the risks of illnesses can be achieved. Functional food can only be provided in the form of food and not as a pill or a capsule. It is part of the usual nutritional behaviou and its effects should be made with the usual amount of consumption (DIPLOCK et al., 1999; KATAN, 1999). Every type of food (natural or industrial) that has one or more bioactive (significantly health protective) material beyond its own nutritive material can be called functional food (SZAKALY és SCHAFFER, 2006). SCHMIDT et al. (2008) believes that functionhal food is that type of food which due to its special nutritive material can hinder or even prevent certain illnesses when used regularly.

Summarising the above points, the objectives of my thesis are as follows:

- According to data found in the technical literature, CLA contents are influenced by
  the season of the year, the keeping, feeding and the breed and so the *first* objective
  of my research work aimed at finding out how the CLA contents of milk changed
  in cattle of different genotypes during lactation.
- 2. The *second objective* of my investigations was to analyse the anti-oxidant effect of the butter containing an increased amount of CLA added to maize-groats.
- 3. As a *third objective* Hungarian big white X Dutch landrace pigs were fed feeds supplemented with ghee the fatty acid compositions of various meat and fat parts were compared.
- 4. Our *fourth objective* was to analyse the changes in the composition of meat parts containing increased amounts of CLA by frying the meat samples in pork fat, sunflower oil and palm oil, i.e., to see what effect the fat used for frying had on the fatty acid composition of the meat sample.

#### 2. MATERIAL AND METHOD

#### 2.1. Changes in the CLA content of milk during lactation

### 2.1.2. The breeds, studied, animal keeping and nutrition

Samples were taken from cows chosen from the cattle stock of Új Élet Mezőgazdasági Termelőszövetkezet (Új Élet Co-operative Farm) at Hencida. The sampling period lasted for one year from March till February. The composition of the breeds was as follows: 50% black Holstein-Friesian, 15% red Holstein-Friesian and 30% Hungarian simmentale cows. During the summer period lasting from 10th May till 15th October the animals mostly ate pasture grasses. When it became necessary they were given 3.5 kg concentrates consisting of 20% lactiferous concentrate, 60% corn and 20% wheat and offal. They were also given phosphorous and calcium supplements and 10-15 kg corn-silage. In winter the animals were fed alfalfa and meadow hay ad libitum and were also given 3.5 kg lactoferious nourishment, 15 kg beetroot slices, 15 kg corn silage and mineral supplements.

### 2.1.3. Methods for milk sampling and storage of the samples

Following the milking into pails 100 cm<sup>3</sup> of equalised milk samples were taken from 3 cows in each group. The milk was cooled down in cold water right away and stored at -25 C<sup>o</sup> until delivery to the laboratory. Afterwards the samples were defrosted and prepared for the analyses at the same time and their fatty acid compositions and CLA contents were determined one after the other in the Institute of Chemistry of the Faculty of Animal Science of the University of Kaposvár.

#### 2.2. The method for preparing the ghee

The butter was melted and heated in a 10 litre pot on an electric cooker. The scum forming while the butter was being heated was removed continuously. When there was no more scum forming the butter was filtered through fluted paper. The strained butter was stored in 100 litre plastic containers. The fat content of the ghee prepared in this way was 98% and its CLA level increased.

### 2.3. Analysis of the anti-oxidant effect of CLA

#### 2.3.1. Mixing the maize and the ghee

The corn was ground to as fine a state as that of flour, to which a 5% amount of ghee of an increased conjugated linoleic acid content was added and the two were mixed together. After thorough mixing and stirring the a 1 cm thick layer of the mixture was poured onto an

aluminium tray covered with tissue-paper where in order to speed up the oxidation process and for reasons of better contact with the air it was continuously mixed with a wooden mixer every day. The mixture was kept at  $20\,^{\circ}$ C for forty weeks.

#### 2.3.2. Taking samples during storage

Samples of the experimental feedstuff were taken every week and the following factors were determined: acid number, peroxide number and the composition of the fatty acids, with the conjugated linoleic acid content included.

#### 2.4. Feeding the feedstuff containing an increased amount of CLA to pigs

#### **2.4.1.** Description of the animal experiments

The pig fattening experiment was conducted at the Experimental farm of the Research Institute for Animal Breeding and Nutrition in Herceghalom. 45 MNF x HL (NNF = Hungarian big white, HL = Dutch Landrace) pigs were involved at individual housing, in three treatments, with 10 animals in each treatment. In the course of fattenning the pigs were fed individually from feeding-troughs in a semi ad libitum manner. Drinking water was provided from nipples. The groups formed, contained both sexes and were of the same genotype. The starting weight of the animals was 36 kg on the average, which reached 94 – 102 kg at the time of slaughter.

The mixing of the maize groats and the ghee was done at the plant of Terményfeltáró Ltd. in Püspökladány. In order to avoid mixing the ghee with other fats, it was mixed with corn-flake flour in a 200 kg capacity mixer. The fat powder produced in this way was put in 40 kg bags. The 520 kg fat powder prepared was directly transported to the Experimental farm of the Research Institute for Animal Breeding and Nutrition in Herceghalom.

Table 1: The compositions and nutrient compositions of the feedstuffs fed during the fattening experiment

	ghee76. ghee33	Control		
Composition, %				
Corn	27,52	27,52		
Barley	34,93	34,93		
Extr. soy 46%	23,70	23,70		
Perfett	-	10,00		
Ghee-corn fat powder	10,00	-		
Lime	1,40	1,40		
MCP	1,40	1,40		
NaCl	0,30	0,30		
L-lysine HCl	0,25	0,25		
Hízó I. 0,5 %	0,50	0,5		
Nutrient content, %				
Dry matter	89,70	89,60		
Crude protein	16,10	17,30		
Ether ee.	6,07	5,50		
Crude fibre	2,05	2,11		
Lysene	2,09	1,83		
Methionine	0,30	0,36		
Threonine	0,59	0,63		

The fat powder containing vegetable oil, which was used to prepare the control feedstuff, was prepared in the fat powder production unit of Abomix Ltd in Herceghalom.

The feed mixes were prepared at the plant of Farmermix Ltd in Zsámbék.

During the experimental periods the animals were fed Hízó I (fattening I) pig feedstuff. The Nourishing substance compositions of the individual feedstuffs are contained in Table 1.

The feed for the animals (ghee76) in the first experimental group contained a ghee supplement for 76 days from the setting of the experiment till slaughtering (Table 2). The animals (ghee33) in the second experimental group were fed a feedstuff supplemented with ghee for 33 days beginning on the 45<sup>th</sup> day from setting the experiment till slaughtering. The feedstuff for the pigs (control) in the control group was supplemented with fat powder made with vegetable oil as a fat supplement.

Table 2: The fatty acid contents of the feed in % of the total fatty acid content

Fatty acid	Feed					
ratty actu	control	ghee76, ghee33				
Caprylic acid8:0	-	0,05				
Lauric acid 12:0	-	0,11				
Myristic acid 14:0	0,10	0,37				
Palmitic acid 16:0	13,70	14,96				
Palmitoleic acid 16:1	0,10	0,19				
Stearic acid 18:0	1,96	2,20				
Oleic acid 18:1	30,30	30,70				
Linoleic acid 18:2	52,40	40,52				
Conjugate linoleic acid 18:2	-	9,18				
Linolenic acid 18:3	1,54	1,53				
Eicoseic acid 20:1	0,10	-				

### The following data were recorded for the evaluation of the experiment:

- The starting and final weights of the animals
- The weights of the animals every month and at changes of the feedstuff regimes
- Daily feed intake
- Slaughter classification
- Fatty acid analysis of tissues samples

#### 2.3.2. Slaughtering the animals, taking samples

The slaughtering of the experimental animals was carried out at the slaughterhouse of the Research Institute for Animal Breeding and Nutrition in Herceghalom. For the fatty acid analysis 200 gr sample was taken from the carcass.

The samples were taken from following locations of the left carcase:

- Long back muscle (musculus longissimus dorsi)
- Crural muscle (musculus semimembranosus)
- Belly fat (panniculus adiposus lateralis)
- Back-fat (panniculus adiposus dorsalis)

#### 2.3.3. The storage of the samples until the analysis

Following the taking of the samples they were transported directly to Department of Biochemistry of the Faculty of Animal Science of the University of Kaposvár where they were stored at -25 C° until the analyses were started. Afterwards the samples were defrosted and prepared for the analyses at the same time and their fatty acid compositions and CLA contents were determined one after the other in the Institute of Chemistry of the Faculty of Animal Science of the University of Kaposvár.

## 2.4. The formation of the fatty acid contents of pork under the effects of being fried in different fats

### 2.4.1. The kinds of fats used for frying

The frying tests were carried out at the Department of Bio-chemistry of the Faculty of Animal Science of the University of Kaposvár. Palmfat, excracted sunflower oils and lard were used. The fatty acid contents of the fats used for the frying tests are seen in Table 3.

Table 3: The fatty acid contents of the fats used in the frying tests

	Lard		Sunflo	ower oil	Palmfat	
	0 min	10 min	0 min	10 min	0 min	10 min
Palmitic acid 16:0	25,03	24,97	6,40	6,32	41,54	43,72
Stearic acid 18:0	13,78	13,94	3,29	3,13	4,44	4,56
Oleic acid 18:1	43,12	42,85	24,13	23,58	40,95	39,18
Linoleic acid 18:2	10,93	10,98	64,45	65,36	10,56	10,17
Linolenic acid 18:3n6	1,05	1,04	0,01	0,06	0,04	0,04
CLA	0,09	0,08	0,01	0,01	Nd	Nd
Arachidonic acid 20:4n6	0,22	0,21	Nd	Nd	Nd	Nd

nd=not determined

### 2.4.2. Description of the process of frying

Slices of an identical size (100 gramm, 2 cm) were cut from the loin and the ham. The meat slices were fried at 160 °C in a DeLonghi deep fryer with a rotary frying pan. They were fried for periods of 1 and 8 minutes.

#### 2.4.3. Storage of the samples until the analyses were started

Following the taking of the samples they were transported directly to Department of Biochemistry of the Faculty of Animal Science of the University of Kaposvár where they were stored at -25 C° until the analyses were started. Afterwards the samples were defrosted and prepared for the analyses at the same time and their fatty acid compositions and CLA contents were determined one after the other in the Institute of Chemistry of the Faculty of Animal Science of the University of Kaposvár.

### 2.5. The analytical – chemical methods applied

The determination of the acid counts and peroxide counts of the samples were carried out according to Magyar Szabvány (Hungarian standard) (MSZ 6830-11:1999).

The determination of the fatty acid compositions and CLA contents of the samples were carried out according to the method of MSZ EN ISO 5509, Animal and vegetable fat and oil. Production of fatty acid metil esther (ISO 5509:2000).

#### 2.6. The statistical analysis of the data

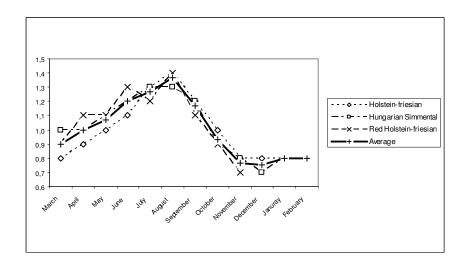
The data were analysed by Microsoft Excell and SPSS 14.0 for Windows (SPSS Inc., Chicago, IL).

#### 3. RESULTS AND THEIR EVALUATION

# 3.1. Changes on the fatty acid composition and CLA content of milk by seasons of the year in cattle of different genotypes

The CLA content reaches its maximum in August (figure 3) and on the average of the breeds it amounts to 1.35%. Between June and September the CLA contents in the milk fat of the milk from each variety exceeded 1.2% and this value decreased rapidly to the values of 0.75 – 0.80% read in the autumn and winter months.

**Figure 1.** The formation of the milk fat content of CLA by seasons of the year in the relative percentage per volume of fatty acid methyl esters



### 3.2. Assessing the inti-oxidant effect of CLA with maize groats

Measured in the relative volume percentage of milk-fat methyl esters the CLA content of butter was 0.56 % before boiling, which increased to 1.68 % in the course of preparing the ghee. When mixed with maize groats the conjugated linoleic acid content of the fatty material obtained was read to be 0.36 %, which means that the maize groats themselves also contained a minimum amount of conjugate linoleic acid.

When analysing the changes in acid counts and peroxide counts we found that over twenty weeks the peroxide counts increased from the beginning value of 7 to 50 and that over the same period the acid counts improved from 5 to 10. Between the 20<sup>th</sup> and 40<sup>th</sup> weeks of the experiment the peroxide counts increased from 50 to 229 and the acid counts grew from 10 to 39. We did not plan to go on with this experiment beyond the 40<sup>th</sup> week since most of the food and feed are utilised over forty weeks and utilisation beyond the 40<sup>th</sup> week is very rare.

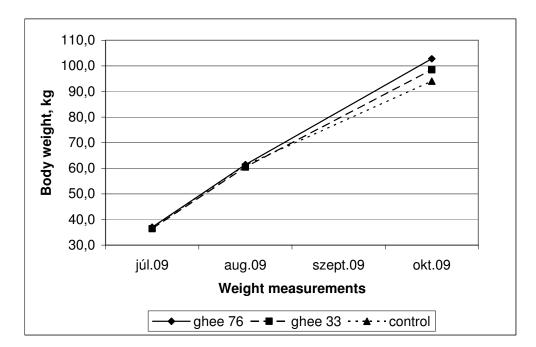
#### 3.3. The effects of increased CLA levels on the fatty acid composition of pork

# 3.3.1. The effects of feedstuff containing increased amounts of CLA contents on the fattening performance of pigs

Body weight gains (figure 2) and feed consumption (figure 3) were measured in the cases of all the three groups. Based on the data recorded the daily weight gains and feed utilisation of the animals were calculated. The data obtained were statistically evaluated.

Studying the data for the weigh gain of the animals we found that in the case of the group fed with ghee for 76 days (ghee 76 group), the average daily weight gain was 901 g. The animals

fed with ghee for 33 days (ghee33 group) had an average daily weight gain of 851 g, while the same for the animals getting a sunflower oil supplement (control group) was 784 g.



**Figure 2:** The weight gains of the experimental animals (kg)

On the basis of the statistical analyses of the data recorded during the fattening experiment we found that there were significant differences between the average daily weight gains of the different groups. The average daily weight gain of group ghee76 proved to be better than that of group control at P<0.01 level of significance. The daily weight gains of the individuals in group ghee33 proved to be better that that of the ones in group control at P<0.05 level of significance.

The average weight of the animals at slaughtering was 98.43 kg. The highest live weights were obtained from the individuals in group ghee76 (102.8 kg), while the lowest values were found with the individuals of group control (94.00kg).

There were significant differences found between the live weights at slaughtering in the different treatments. The slaughtering live weights of the individuals in the group (ghee76) receiving feeds containing a high level of CLA for 76 days were significantly higher at P<0,01 than those of the pig group (control) fed with feeds containing a sunflower iol supplement. The live weights of the pigs in the group (ghee33) feeding on sunflower oil for

33 days and then on feeds containing a high level of CLA were significantly higher at P<0,05 level in comparison to those of the control group.

On the basis of the above it can be claimed that under the influence of the experimental feedstuff fattening pigs reach a suitable slaughtering live weight.

Studying the data for feed consumption of the animals we concluded that during the experiment the average feed uptake of the animals (figure 10) in group ghee76 was 2.39 kg in the two phases of the fattening period. In the case of the ghee33 group the average daily feed uptake of the animals was 2.38 kg while in the case of the control group this figure is 2.44 kg.

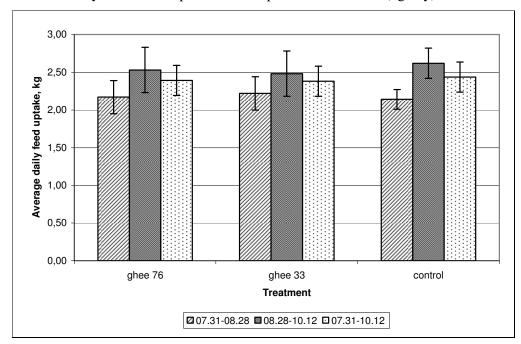
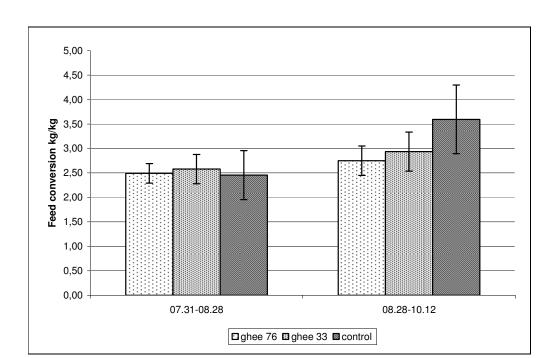


Figure 3: The daily feed consumption of the experimental animals (kg/day)

On the basis of the data obtained we concluded that the difference between the daily feed uptakes of groups ghee76 and ghee33 was significant at P<0,001 level of probability. At the P<0,001 level of probability, the difference between the individuals in groups ghee76 and control were also found to be significant.

From the data recorded during the experiment we calculated the average feed conversion values for the experimental animals (figure 4) and found they were more favourable in the case of the animals feeding on feedstuff containing a high level of CLA.



**Figure 4:** The average feed conversion of the experimental animals (kg/kg)

This also means that the individuals in group ghee33 produced 1 kg live weight gain by consuming an average of 2.80 kg feedstuff. This value is 2.65 for the individuals in group ghee76 and the same is 3.11 kg for the animals in group control.

As a result of the statistical analysis conducted with the help of the calculated figures the feed conversion of group ghee33 was found to be significantly better (P<0.01) than the feed conversion ratio of control and at P<0.05 level of significance it exceeded that of the individuals in group ghee76 as well.

## 3.3.2. The effects of the feedstuff containing an increased level of CLA on the fatty acid composition of pork and lard

The average CLA content of the *loin-chop* samples expressed in the relative percent by weight of fatty acid methyl esters of the group eating feed mixed with ghee for 33 days was found to be 0.11%, while the same value for the animals eating feed mixed with ghee for 76 days was 0.17 and for the control group eating sunflower oil it was 0.08%. On the basis of the significance assessment the CLA contents of the animals fed with ghee-supplemented feedstuff for both 76 an 33 days was at 0.1% of probability significantly higher than that of the control group and even that of the animals eating feed mixed with ghee for 76 days was at

0.1% of probability significantly higher than that of the animals eating the same feed stuff for 33 days only.

**Table 5.** The average fatty acid composition of loin-chop samples

Fatty acids	Ghee76		Ghee33		Control				
	Fatty acid methil-esther %								
	Mean	SD	Mean	SD	Mean	SD			
n	10		1	10		10			
10:0	0,09	0,01	0,07	0,01	0,08	0,01			
12:0	0,10	0,02	0,08	0,01	0,07	0,02			
14:0	1,58	0,11	1,31	0,14	1,20	0,11			
14:1	0,05	0,02	0,07	0,03	0,04	0,01			
15:0	0,11	0,05	0,17	0,11	0,08	0,03			
16:0	25,45	0,94	24,24	1,02	23,97	0,98			
16:1	2,50	0,40	2,38	0,31	2,28	0,28			
17:0	0,53	0,08	0,45	0,18	0,34	0,06			
18:0	14,54	1,62	13,75	0,85	13,43	0,81			
18:1t	0,65	0,45	0,50	0,42	0,30	0,09			
18:1c	43,31	1,45	42,64	1,50	40,77	1,38			
18:2	8,10	0,78	10,90	1,46	13,93	1,81			
20:0	0,30	0,05	0,34	0,05	0,36	0,17			
18:3 ω6	0,05	0,02	0,05	0,02	0,05	0,01			
20:1	0,79	0,10	0,75	0,07	0,73	0,08			
18:3 ω3	0,31	0,04	0,30	0,04	0,30	0,05			
CLA 18:2 c9, t11	0,17	0,03	0,11	0,02	0,08	0,01			
20:2	0,33	0,06	0,47	0,07	0,59	0,07			
20:3 ω6	0,18	0,07	0,21	0,06	0,21	0,05			
23:0	0,05	0,02	0,06	0,01	0,06	0,01			
20:4 ω6	0,76	0,44	1,15	0,40	1,13	0,33			
SFA	42,75		40,47		39,59				
UFA	57,25		59,53		60,41				
PUFA	9,9		13,08		16,29				
ω-6	0,99		1,41		1,39				
ω-3	0,31		0,3		0,3				
ω-6/ω3	3,19		4,70		4,63				

The CLA content of the *pork-chop* samples of the group eating feed mixed with ghee for 76 days (ghee 76) was (0.21 %) at 0.1 level of probability was significantly higher than that of the group eating it for 33 days (ghee 33) or that of the control group (0.11, 0.10 %). There were no significant differences found in the CLA contents between the ham samples taken from the ghee 33 and the control group.

**Table 6.** The average fatty acid composition of pork-chop samples

Fatty acids				Ghee33		Control			
	Fatty acid methil-esther%								
	Mean	SL	)	Mean	SD	Mean	SD		
n	10			10		1	0		
10:0	0,09	0,0	1	0,08	0,01	0,08	0,02		
12:0	0,11	0,0	2	0,10	0,04	0,08	0,05		
14:0	1,63	0,1	9	1,20	0,48	1,13	0,16		
14:1	0,09	0,0	4	0,09	0,03	0,04	0,02		
15:0	0,15	0,0	8	0,24	0,18	0,07	0,02		
16:0	24,36	0,4	9	23,92	1,39	22,55	0,92		
16:1	2,85	0,3	6	2,58	0,40	2,53	0,41		
17:0	0,58	0,1	1	0,44	0,09	0,38	0,11		
17:1	0,44	0,0	8	0,34	0,09	0,27	0,08		
18:0	12,43	1,0	4	11,12	3,47	11,46	0,88		
18:1t	0,37	0,1	1	0,34	0,06	0,29	0,09		
18:1c	43,18	3,1	7	42,14	4,94	40,41	3,89		
18:2	10,09	2,0	8	13,02	2,56	16,44	3,60		
18:3 ω6	0,08	0,0	3	0,08	0,03	0,12	0,21		
20:1	0,76	0,1.	2	0,71	0,11	0,62	0,21		
18:3 ω3	0,37	0,0	8	0,31	0,03	0,33	0,13		
CLA 18:2 c9, t11	0,21	0,0	6	0,11	0,01	0,10	0,05		
20:2	0,33	0,0	4	0,47	0,07	0,62	0,07		
22:0	0,05	0,0	3	0,05	0,02	0,05	0,02		
20:3 ω6	0,27	0,0	7	0,37	0,14	0,27	0,10		
23:0	0,06	0,0	1	0,05	0,01	0,05	0,01		
20:4 ω6	1,50	0,6	0	2,58	1,42	2,11	1,23		
SFA	39,46			37,20		35,85			
UFA	60,54			62,80		64,15			
PUFA	12,85			16,94		19,99			
ω-6	1,86			3,03		2,50			
ω-3	0,37			0,31		0,33			
ω-6/ω-3	5,03			9,77		7,57			

The CLA content of the *belly fat* (0.26%) of the 76 group was at 0.1% level of probability significantly higher than that of either the ghee33 (0.19%) or the control group (0.11%). The CLA content of the belly fat of the ghee33 group was at 0.1% level significantly higher than that of the control group.

**Table 7.** The average fatty acid composition of belly fat samples

Fatty acids	Ghee76			Ghee33		Kontroll			
	Fatty acid methil-esther %								
	Mean	SL	)	Mean	SD	Mean	SD		
n	10			10		1	10		
10:0	0,09	0,0	1	0,08	0,01	0,08	0,01		
12:0	0,13	0,0	2	0,10	0,01	0,07	0,01		
14:0	1,83	0,5	2	1,58	0,16	1,32	0,12		
14:1	0,06	0,0	1	0,04	0,02	0,02	0,00		
15:0	0,14	0,0	3	0,10	0,03	0,05	0,02		
16:0	24,74	0,8	1	23,61	1,17	22,59	1,44		
16:1	3,10	0,3	6	2,60	0,35	2,45	0,33		
17:0	0,59	0,0	7	0,48	0,09	0,37	0,08		
17:1	0,50	0,0	5	0,39	0,10	0,30	0,07		
18:0	11,71	0,7	'4	11,78	0,51	11,56	0,74		
18:1t	0,45	0,0	5	0,34	0,05	0,28	0,08		
18:1c	45,37	1,2	4	43,43	1,57	42,61	1,54		
18:2	8,61	0,9	9	12,57	1,74	15,30	1,71		
20:0	0,34	0,0	7	0,41	0,14	0,51	0,13		
18:3 ω6	0,05	0,0	1	0,04	0,01	0,04	0,01		
20:1	0,82	0,1	2	0,76	0,08	0,74	0,10		
18:3 ω3	0,43	0,0	5	0,45	0,06	0,40	0,05		
CLA 18:2 c9, t11	0,26	0,0	2	0,19	0,03	0,10	0,02		
20:2	0,37	0,0	5	0,57	0,09	0,69	0,08		
20:3 ω6	0,11	0,0	2	0,11	0,02	0,11	0,02		
23:0	0,06	0,0	1	0,07	0,01	0,06	0,01		
20:4 ω6	0,25	0,0	4	0,30	0,04	0,35	0,04		
SFA	39,63			38,21		36,61			
UFA	60,37			61,79		63,39			
PUFA	10,08			14,23		16,99			
ω-6	0,41	1		0,45		0,5			
ω-3	0,43	1		0,45		0,4			
ω-6/ω-3	0,95			1,00		1,25			
		1			I .	1			

The CLA content of the *back fat* samples (0.30 %) was at 0.1 % level of probability significantly higher in the ghee76 group than either in the ghee33 group (0.21 %) or the control group (0.17 %). The CLA content of the back fat of the ghee33 group was at 0.1 % level of probability significantly higher than that of the control group.

**Table 8.** The average fatty acid composition of back fat samples

	Ghee76		Ghee33	1	Kontroll					
Fatty acids		Fatty acid methil-esther %								
	Mean	SD	Mean SD		Mean	SD				
1	n 10	)	1	10		.0				
10:0	0,10	0,01	0,08	0,01	0,06	0,01				
12:0	0,16	0,02	0,11	0,01	0,07	0,01				
14:0	2,09	0,19	1,55	0,15	1,18	0,10				
14:1	0,07	0,01	0,04	0,01	0,02	0,01				
15:0	0,16	0,03	0,10	0,01	0,06	0,02				
16:0	25,03	1,15	23,39	1,30	22,24	1,25				
16:1	2,37	0,37	1,91	0,32	1,76	0,28				
17:0	0,75	0,06	0,55	0,11	0,42	0,08				
17:1	0,60	0,16	0,43	0,11	0,31	0,08				
18:0	13,73	1,75	13,46	1,08	12,96	1,52				
18:1t	0,55	0,05	0,39	0,05	0,27	0,05				
18:1c	40,88	1,04	39,41	1,11	38,09	1,65				
18:2	10,71	1,33	15,44	1,96	19,15	2,08				
20:0	0,30	0,04	0,38	0,06	0,47	0,03				
18:3 ω6	0,05	0,01	0,04	0,01	0,04	0,01				
20:1	0,76	0,10	0,80	0,18	0,76	0,10				
18:3 ω3	0,55	0,07	0,55	0,07	0,54	0,15				
CLA 18:2 c9, t11	0,30	0,05	0,21	0,03	0,17	0,12				
20:2	0,43	0,07	0,67	0,10	0,89	0,14				
20:3 ω6	0,11	0,02	0,12	0,02	0,13	0,01				
23:0	0,08	0,01	0,08	0,01	0,07	0,01				
20:4 ω6	0,22	0,05	0,29	0,04	0,35	0,07				
SFA	42,40	Ì	39,70		37,53					
UFA	57,60		60,30		62,47					
PUFA	12,37		17,32		21,27					
ω-6	0,38		0,45		0,52					
ω-3	0,55		0,55		0,54					
ω-6/ω-3	0,69		0,82		0,96					

## 3.4. The formation of the fatty acid composition of pork as a result of being fried in different kinds of fat

Estimation of frying datas it was determined that higher (0.13%) CLA content of pork was spoiled (60-70%) except in case of swine fat cooking, because it is extremly sensitive for oxidation and heating. Swine fat has higher (0.09%) CLA content than plant oil, and this prevents the meat's original CLA content. Cooking in swine fat did not have significant effect on fatty acid composition of meat. Low level of palmitic acid contect of sunflower oil (6.40%) decreased for half part of palmitic acid content of pork (24.13%) and it produced cooked meat with decreased oil acid content. Contrary of above, linoleic acid content of fried meat was increased in different folds compare to pork. If it was fried in sunflower oil with high level linoleic acid increased (51.52%) the linoleic acid content in fried pork. The linoleic acid content of the high level CLA pork increased four times (48.59%) to the crude meat (16.59% and 12.32%). The high palmitic acid content of palm fat (41.54%) increased with 60% the palmitic acid content in fried pork, low stearic acid (4.44%) and linoleic acid content (10.56%) decreased the stearic and linoleic acid content of crude meat.

#### 4. CONCLUSIONS, RECOMMENDATIONS

1. While studying the changes in the fatty acid compositions and CLA contents of milk according to seasons of the year it was concluded that most of the saturated fatty acids reached minimums in the summer months and showed maximum values in the winter and early spring months. In contrast, the concentration of unsaturated fatty acids, including CLA as well, showed maximum values in the summer months and reached their minimums in all the cases in the winter and early spring months. As regards the trends these findings are in line with the ones found in the technical literature and even as regards the maximum values the deviations from the data in the technical literature are minimal. On the basis of the data obtained it can be established that irrespective of the breed the milk milked in summer contained considerably more linoleic, linolenic and oleic acids as well as CLA than the milk obtained in winter and early spring, thus as regards the preservation of human health it was more suitable for human consumption. As the production of the animals occurred under totally identical conditions – they consumed mostly pasture grass in summer and hay and silage in winter the higher CLA level in the milk in summer is likely to be explained with the

higher unsaturated fatty acid or possibly CLA content of the pasture grass or effects of the ultraviolet rays of sunshine.

- 2. While studying the anti-oxidant effect of CLA it was established that out of all the multiple unsaturated fatty acids it was the conjugated linoleic acid that was the most sensitive to oxidation and so had the most powerful anti-oxidant effect. Due to the increased CLA content the amounts of (the essential for human beings9 linoleic acid and (the semi essential) linolenic acid showed hardly any changes duringthe first week of storage and as regards their proportions the changes were negligible in comparison to conjugated fatty acids even after week 20 of storage. According to our findings the butter containing an increased CLA content (ghee), due to its considerable anti-oxidant properties, protects the oxidation sensitive components of food and feedstuff.
- 3. As a result of the fattening experiment it can be concluded that the experimental feedstuff increased both the average daily weight gains and average daily feed conversion ratios of the animals. In the case of all the samples we analysed the CLA contents of the samples from the animals eating feedstuff mixed with ghee were the highest and those from the animals not eating ghee were the lowest. The group receiving feedstuff mixed with ghee showed values between those of the group eating feedstuff mixed with ghee for 76 days and the control group. The CLA contents of the ghee76 group in the cases of all the four samples were significantly higher at 0.1 % of probability than those of either the ghee33 or the control groups. With the exception of the ham the ghee33 group showed significantly higher contents of CLA at 0.1% level of probability than the samples taken from the control group. The only significant difference between the ghee33 group and the control group was observed in CLA contents. From the above facts we can conclude that in the case of pigs, feeding with feedstuff enriched with ghee for 76 days increased the CLA contents of fibres significantly but as regards ham 33 days seem few to bring about significant increases. Our experiment shows that it is worth feeding pigs with feedstuff containing increased CLA amounts for longer than 33 days since as a result of feeding this feedstuff to pigs for 76 days the CLA contents in all the samples analysed were significantly higher not only in comparison to the control group but to the ghee33 group as well. As for the total number of samples analysed it was the back-fat that contained the most and the pork-chop contained the least CLA. At the same time the ham showed similar values

to the pork chop and the values for the belly fat were very near the values for the backfat. Although these analyses did not cover this aspect but it is likely that there may be significant differences as regards the CLA contents of the different parts of the body of the pig.

As regards the four samples it was the body tissue of the animals feeding on feedstuff containing a sunflower oil supplement for 76 days that contained the highest level of linoleic acid. In the case of linoleic acid we did not find as significant differences between the different parts of the body as in the case of CLA contents. As regards the ghee 76 group the four body parts showed values for linoleic acid contents of between 8.10 % and 10.71 %. In the case of the ghee33 group these values ranged between 10.90 % and 15.44 % and these values were between 13.93 % and 19.15 % in the control group. In the case of arachidonic acid the situation is far from being as clear as above. On the one hand the back-fat and belly-fat contained much less of this fatty acid than the ham or the pork-chop, and on the other hand some trend towards a change can only be seen in the two fat samples. Due to the very small concentrations, however, it is hardly possible to draw any conclusions. When analysing the fatty acids containing short chain-length carbon cycles we concluded that the amounts of capric, laurinic and miristic acids were in most cases significantly higher in the case of the group eating feedstuff mixed with ghee for 76 days than the same values for the control group, which can most likely be linked to the fact to the fatty acids with considerably higher short and medium carbon atom counts in the ghee. The amounts of palmitic acid, which accounts for almost 25 % of all fatty acids were for all the samples from the ghee76 group significantly higher than those of the control group and with the exception of the back and belly fat samples, the same can be said of the stearic acid contents of the pork chop and ham samples. As regards oleic acid accounting for almost 40% of all fatty acids, in the cases of all the samples the 76 group yielded the highest and the control group had the lowest values, which was surprising as the oleic acid content of the sunflower oil used to supplement the feedstuff for the control group contains more oleic acid than ghee does. In this latter case corn that constituted the biggest part of the feedstuff had more influence on the fatty acid composition of the body tissues of the pig than ghee or sunflower oil given as supplements.

4. As a result of frying, with the exception of frying in pork fat, most of the increased CLA content of pork deteriorates as it is highly sensitive to oxidation and heat treatment. The relatively high CLA content of pork fat – in comparison to those of vegetable oils – provides some protection for the original CLA content of pork. Frying pork in pork fat does not change the meat's fatty acid composition considerably. The situation is quite different in the cases of the two vegetable oils tested in this experiment. It was concluded that the low palmitic acid content of vegetable oil decreases the palmitic acid content of raw meat considerably and the also low oleic acid content of vegetable oil results in fried meat with a reduced oleic acid content. In contrast, however, the extremely high linoleic acid content of vegetable oil will double, what is more, triple the linoleic acid content of fried meat in comparison to the original values of raw meat. The high palmitic acid content of the palm fat increases while its low stearic and linoleic acid contents decrease the original stearic and linoleic contents of fried meat. By testing the nutrient compositions of raw and fried meat it was established that the transition if fats was statistically verifiably higher (P<0,05) when the meat was baked. Our results demonstrate that as regards fried meat we are far from consuming the food having the fatty acid content that would be expectable from the original raw material, insomuch as the fatty acid composition of fried meat differs considerably from the original fatty acid composition of meat as it is influenced by frying to a great degree. It follows from the above facts that the fatty acid composition of meat can be influenced favourably for human consumption or otherwise depending on the composition of the fat used for frying.

#### 5. NEW AND RECENT SCIENTIFIC RESULTS

- 1. I determined the fatty acid composition and the changes in the fatty acid content of the milk The amount of the saturated fatty acids dropped to a minimum level during the summer months, and reached a maximum during winter and in early spring. The amount of oleic, linoleic, and linolenic acid and conjugated linoleic acid was the highest in summer, which was probably due to the different type of feeding in winter and in summer.
- 2. I proved during a 40-week long experiment the antioxidant effect of CLA, in which the acid number and the peroxid number of the corngris that was mixed

with a ghee of 5% increased CLA content and stored at roomtemperature did not surpass the standard value, and apart from the CLA the essential fatty acids were not damaged significantly.

- 3. The ghee with high conjugated linoleic acid content is appropriate to produce high fat content feed for pigs.
- 4. All ready with a 33-day long ghee feed supplement the conjugated linoleic acid content of the pork will be significantly increased. We found that the ghee feeding to the 76 days was the most effective.
- I analysed the effect of feeding high CLA-content (conjugated linoleic acid) feed on the composition of fatty acids in pork. As an effect of feeding ghee-enriched feed, the CLA content significantly increased.
- 6. With frying experiments I proved that the original acid content of the pork was significantly affected by the acid content of the frying oil. I established the fact that apart from frying in swine fat, the original CLA content was significantly damaged when being fryid in sunflower oil.

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