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Dietary ecologic camelina oil – a beneficial source of n-3 PUFA in muscle tissue and health status in finishing pigs

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MIHAELA HABEANU[1,2], VERONICA HEBEAN[1], IONELIA TARANU[1], MARIANA ROPOTA[1], NICOLETA LEFTER[1], DANIELA MARIN[1,2].
[1]National Research-Development Institute for Animal Biology and Nutrition. Balotesti. Romania. Calea Bucuresti nr.1, 077015 Balotesti, Ilfov, ROMANIA
[2]Postdoctoral School for zootechnical biodiversity and food biotechnology based on the eco-economy and the bio-economy required by eco-sanogenesis, ROMANIA.
e-mail: mihaela.habeanu@ibna.ro;

Abstract

The objective of this study was to highlight the positive effects of the dietary ecologic camelina oil providing n-3 PUFA on fatty acid (FA) composition of total lipids of Longissimus dorsi, LD and Semitendinosus, ST muscles as well as its effects on blood plasma composition in finishing pigs. The experiment was carried for 30 days on 20 Large White finishing pigs randomly assigned to two isoenergetic and isonitrogenous diets: the control group (sunflower oil diet) received a diet including conventional ingredients based on sunflower oil (containing predominantly linoleic FA (C18:2n-6 = 64.62%) and the experimental group with supplement in n-3 PUFA by addition of camelina oil receiving a diet including ecological ingredients. Gas chromatography was used to determine the detailed composition in FA in muscle tissues (Longissimus dorsi and Semitendinosus). At the end of feeding trial blood samples were aseptically collected by jugular venipuncture from 6 animals of each group in order to determine the chemical composition (glycaemia, lipids and protein profile). Plasma biochemical analyses were performed using Analyser BS -130. Our results showed that the incorporation of α linolenic FA into muscle tissue was significantly higher following the dietary addition of camelina oil whatever the type of muscle. (by a factor of 2.83 on LD muscle, P<0.0001, and 1.40 on ST muscle, P<0.02, vs. control group). The reduction of C18:2n-6 : C18:3n-3 ratio (3.44 times in LD muscle and 2.13 times in ST muscle, P<0.0001 vs. control group) was important. The addition of n-3 PUFA to the experimental diet showed an accumulation of long- chain FA (DHA) known for its beneficial effects on human health. In plasma, the higher content of n-3 PUFA in the camelina oil influenced particularly the lipid composition. The concentration of cholesterol and triglycerides was decreased. Our results confirm that the camelina oil by its content in n-3 FA, alters significantly the fatty acids composition in the muscle tissue. Moreover, the results obtained in this study could be used for a better understanding of the biochemical processes in finishing pigs for estimating their physiological status.

Key words: Camelina oil, Ecologic, n-3 FA, Health status, Finishing pigs.

Introduction

N-3 fatty acids are key elements of polyunsaturated fats due to their beneficial implication on human health (Bauchart et al., 2010, Mahecha et al., 2009, Riediger et al., 2009). The health effects of long-chain PUFA (polyunsaturated fatty acids) are diverse and nutritional studies continue to demonstrate the important benefits of n-3 PUFA consumption (Chapkin et al., 2008). Since the use of a health claim (beneficial effects) on labels for foods containing n-3 PUFA has been approved, food companies are now mobilising to incorporate these fatty acids into a range of novel commercial foods in order to promote the wider public consumption of these bioactive compounds. In our diets there are two types of n-3 fatty acids: α–linolenic FA (ALA) found in vegetable oils (linseed, camelina, hemp, spinach, lettuce, etc.) and eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids, found especially in fat fish (Riediger et al., 2009). The body partially converts ALA to EPA and DHA. Nutrition is

the main factor which can affect the storage and structure of lipids and fatty acids in meat even if other factors as genotype, age, sex, environment can have an effect on their content (Kloareg et al., 2007). It was also stated that the human modern diets are deficient in n-3 FA and the n-6: n-3 ratio is 10-20:1 (Stoll, 2001). The studies focused on aspects of evolution in our food regimes are carried on the type and quantity of essentially fatty acids and antioxidant content of food (Dunkin et al., 1996).

In 2008, at FAO initiative, the linkage between nutrition, food, health and biodiversity was recognized establishing key indicators of nutrition (e.g. food composition) that can contribute to promoting and preserving dietary biodiversity and food security. This situation is due manly to insufficient data on the nutritional value of local resources but also to the lack of the methods for obtaining, analysing and using biodiversity data in studies related to intake and food composition. The requirements of consumers are oriented toward using of fresh food, without antibiotics, free of pesticides and other harmful substances, for developing a sustainable environment.

The objective of this study was to highlight the positive effect of the dietary ecologic camelina oil as sources of n-3 PUFAs on lipid fatty acid (FA) composition of *Longissimus dorsi* and *Semitendinosus* muscles as well as its effects on blood plasma composition in finishing pigs. The camelina oil obtained by cold pressing was included in the experimental diet due to its content in n-3 PUFA (especially, C18:3n-3 FA). This ingredient can be a key source for altering the structure of fatty acids in the muscle tissues. On the other hand, camelina (*Camelina sativa*) can be considered a low–input feed ingredient requiring small quantities of water and a lower fertilization than other oleaginous species. Furthermore, this source rich in n-3 PUFA could be an opportunity for the nutritional researches and feeding technology (Habeanu et al., 2009, Hebean et al., 2008). The effects of camelina oil on fatty acids composition in muscle tissue could be reinforced by addition of peas with 11.54% level of C18:3n-3.

The knowledge on food composition is limited, especially on the ecologic food but also on camelina effects on lipid metabolism, which had provided us the opportunity to initiate the further range research on this topic.

Material and methods

Animals and diets

The feeding trial was carried out for 30 days on 20 Large White finishing pigs assigned to two groups (10 pigs/ group): *control group* (sunflower oil) – the animals were fed with conventional ingredients and sunflower oil used as oleaginous source (64.62% C18:2n-6 fatty acids); *experimental group* (camelina oil) the animals were fed with ecologic ingredients and camelina oil (3%) was used as a source of n-3 PUFAs (12.84% α linolenic FA).

The compound feed formulation was isoenergetic and isonitrogenous (table 1). Food delivery was "ad libitum" with permanent access to water and similar experimental conditions.

Table 1. Ingredients and chemical composition of the diet (%)

Ingredients	Sunflower oil	Camelina oil
Barley	22.85	37.90
Wheat	48.00	20.00
Peas	-	10.00
Full fat soy	-	13.00
Soybean meal	7.00	5.00
Sun flower meal	14.50	7.00

Camelina oil	-	3.00
Sun flower oil	3.50	-
Methionine	-	-
Lysine	0.30	0.10
Ca Carbonate	1.75	1.70
Mono calcium phosphate	0.80	1.00
NaCl	0.20	0.20
Choline	0.10	0.10
Premix vitamins and minerals	1.00	1.00
Analyses		
EM (Kcal /kg)	3025	3192
PB (%)	15.57	15.55
Crude Lysine (%)	0.82	0.88
Digestible Lysine (%)	0.69	0.72
Crude M+Cys (%)	0.54	0.52
Digestible M+Cys (%)	0.45	0.41
Calcium (%)	0.89	0.92
Phosphor (%)	0.60	0.63
Cellulose (%)	6.83	6.42
C18:3n-3	4.22	4.83
C18 :2n-6	55.02	52.51
C18 :2n-6 : C18:3n-3	13.66	10.86
DHA	0	0.10

Measuring and analyses

Weende analysis

The crude protein of the diet and of the muscles was determined using a semiautomatic classical Kjeldahl method using a Kjeltek auto 1030 – Tecator (SR EN ISO 5983-2, 2009 and SR ISO 973, 2007, respectively). The fat was extracted using an improved version of the classical method by continuous extraction in solvent, followed by fat measurement with Soxhlet after solvent removal (SR ISO 6492, 2001 for the diet and SR ISO 1444, 2008 for the muscles). The crude fibre was determined with a classical semiautomatic Fibertec-Tecator method (SR EN ISO 6865, 2002) and the ash by calcinations' at 5500 until constant mass (SR EN ISO 2171, 2010 for the diet and SR ISO 936, 2009 for the muscle). The nitrogen-free extractives (NFE) were calculated from the formula: NFE = DM - (CP + EE + CF + Ash). The metabolizable energy (ME) was calculated with regression equations developed by the „Oskar Kellner" Institute of animal nutrition: ME = 5.01 × DP + 8.93 EE + 3.44 CF + 4.08 DNFE.

Fatty acid analysis

The muscle tissue (*Longissimus dorsi* and *Semitendinosus*) and ingredients FA composition was determined by gas chromatography. After lipid extraction from the samples, the FAs were transformed into methyl esters by transmethylation, and the components were separated in the capillary chromatograph column. The fatty acids were identified by comparison with blank chromatograms and were subsequently determined quantitatively as percent for 100 g fat. SUPELCO 37 component FAME Mix was used; 10 mg/ml as standard solution of methylated fatty acids and also Soybean Oil and Sunflower Oil; SUPELCO, as reference material was used. Perkin Elmer-Clarus 500 gas chromatograph fitted with a system of injection into the capillary column (splitting ratio about 1:100), with programmed chromatographic column oven heating was used; the system was fitted with flame ionization detector (FID) and column of high polarity stationary capillary separation (SGE forte GC

Capillary Column BPX70, 60m L; 0.25mm inner diameter, 0.25um.gros.film). We used hydrogen as carrier gas and the air oxygen as burning gas. The methylated fatty acids from the sample were separated according to chain length, to the level of unsaturation and to the geometry of the double bonds. A control sample (n-hexane) and a reference sample (CRM) were analysed in parallel with the analysed sample (or batch of samples).

Camelina oil analysis

The camelina seeds were obtain from ecological culture. Cold pressing was used as ecologic method for oil extraction. The oils extracted with this method are better in terms of content and they preserve unaltered the nutritive quality of the seeds. The yield is 38-45% oil. This procedure is ecologic because isn't used the chemical substances and PUFA are not altered. Camelina oil has the advantage to be alternative source of n-3 PUFA to linseed, the production for this ingredient being higher in many countries including Romania.

Statistical analysis

The results were expressed as mean values and SEM. The data were submitted to an analysis of variance using SPSS 12 statistical software, 2003 (ANOVA – general linear model, GLM). Effects were considered significant at $P \leq 0.05$. At 10% we consider that there is a tendency the results to be influenced by the diet. Values for each fatty acid or for a family of fatty acids were expressed in relative (in % of total fatty acids).

The Pearson correlation coefficient was determined for nutritive substances on the muscle tissue.

Results and discussion

Chemical analyses

Table 3 shows fatty acids composition of the dietary ecologic ingredients.

Table 3. Fatty acids composition for ecologic ingredients (%)

Fatty acids	Sunflower meal	Full fat soybean	Camelina oil	Peas	Barley	Wheat
C14:0 (myristic)	0.09	0.13	0.11	0.54	0.41	0.30
C16:0 (palmitic)	7.34	12.65	6.27	14.53	24.77	22.69
C18:0 (stearic)	2.40	3.80	2.09	3.12	0.17	1.26
C16:1 (palmitoleic)	0.11	-	-	-	1.29	0.42
C18:1cis-9 (oleic)	23.63	18.24	17.93	27.80	13.30	13.46
C18:2n-6 (linoleic)	63.97	55.25	22.10	42.16	55.11	56.75
C18:3n-6 (γ linolenic)	-	-	31.61	-	-	-
C18:3n-3 (α linolenic)	0.30	0.19	12.84	11.54	4.84	5.13
C20:4n-6 (arachidonic)	-	0.13	0.90		-	-
C22:6n-3 (docosahexaenoic)	-	-	3.32	-	-	-

Camelina oil had a high content in PUFA, especially in γ linolenic FA (31.61%) and also α linolenic FA (12.84%). The C18:2n-6 : C18:3n-3 ratio was 1.72, which was a supplementary advantage for using it. The peas also increase the dietary content in n-3 PUFA by its content in C18:3n-3 close to camelina oil (11.54%). Many data show the uniqueness of DHA due to the fact this FA alters significantly the main characteristics of the cell membrane, without minimising the importance of biological effects of the other n-3 PUFA (Stillwell & Wassall, 2003). In part, due to the number of *cis* double bonds, DHA is sterically incompatible with sphingolipid and cholesterol and, therefore, appears to alter lipid shelve behaviour (Stillwell & Wassall, 2003).

Weende composition on Longissimus dorsi and Semitendinosus muscles

Table 4 shows the gross chemical composition of the two types of muscles *(Longissimus dorsi* and *Semitendinosus)*. There were not significant differences between the groups

whatever the nutritive substance determined (P>0.05). The level of protein was more important in ST muscle than LT muscle while the level of fat was reduced, the correlation between the two being negative (Pearson correlation = -0.97 for *Longissimus dorsi* and –0.90 for *Semitendinosus*).

Table 4. Weende composition of muscular tissue (%)

Muscles	Items	Sunflower oil	Camelina oil	*SEM*	*P*
Longissimus dorsi	Dry matter	87.63	88.59	*0.23*	*0.18*
	Crude protein	65.47	61.54	*2.50*	*0.47*
	Ether extractives	18.32	20.40	*3.00*	*0.74*
	Gross ash	4.38	4.22	*0.17*	*0.66*
Semitendinosus	Dry matter	88.59	88.71	*0.05*	*0.32*
	Crude protein	69.98	69.04	*1.07*	*0.68*
	Ether extractives	11.61	11.13	*1.33*	*0.86*
	Gross ash	4.79	4.93	*0.12*	*0.59*

Fatty acids composition of the muscles (%)

The analysis of fatty acids extracted from total lipids of LD and ST muscles generally showed (table 5) significant differences for n-3 PUFA.

Table 5. Fatty acids composition in *Longissimus dorsi* and *Semitendinosus* muscles

Fatty acids**	*Longissimus dorsi**				*Semitendinosus**			
	Sunflower oil	Camelina oil	*SEM*	*P*	Sunflower oil	Camelina oil	*SEM*	*P*
C14:0 (myristic)	1.71	1.68	*0.06*	*0.87*	1.63	1.78	*0.15*	*0.64*
C16:0 (palmitic)	26.89	25.82	*0.51*	*0.32*	25.75	26.15	*0.87*	*0.83*
C16:1 (palmitoleic)	2.42	2.13	*0.12*	*0.26*	2.94	2.91	*0.14*	*0.92*
C18:0 (stearic)	13.09^{T}	12.31^{T}	*0.22*	*0.07*	11.48	10.53^{T}	0.29^{T}	*0.10*
C18:1cis-9 (oleic)	36.56^{a}	33.22^{b}	*0.78*	*0.02*	36.34	33.95	*0.88*	*0.19*
C18:2n-6 (linoleic)	16.39	17.39	*0.53*	*0.38*	17.54	17.89	*0.60*	*0.78*
C18:3n-6 (γ linolenic)	0.28^{a}	3.14^{b}	*0.46*	*<0.0001*	0.34^{a}	2.65^{b}	*0.37*	*<0.0001*
C18:3n-3 (α linolenic)	0.40^{a}	1.53^{b}	*0.19*	*<0.0001*	0.43^{a}	1.03^{b}	*0.14*	*0.02*
C20:4n-6 (arachidonic)	1.05	0.92	*0.17*	*0.73*	2.03	1.56	*0.21*	*0.29*
C22:6n-3 (docosahexaenoic)	0^{a}	0.21^{b}	*0.03*	*<0.0001*	0^{a}	0.17^{b}	*0.03*	*0.004*
Sum SFA	41.70	39.81	*0.64*	*0.15*	38.86	38.45	*0.87*	*0.82*
Sum MUFA	39.19^{a}	35.17^{b}	*0.83*	*0.027*	39.28	36.86	*0.88*	*0.18*
Sum PUFA	19.11^{a}	24.49^{b}	*1.19*	*0.015*	21.46	24.23	*0.98*	*0.17*
Sum n-6	17.74^{a}	21.46^{b}	*0.88*	*0.027*	19.90	22.11	*0.85*	*0.21*
Sum n-3	0.39^{a}	1.74^{b}	*0.22*	*<0.0001*	0.42^{a}	1.19^{b}	*0.16*	*0.01*
Linoleic/ α linolenic	42.81^{a}	12.44^{b}	*5.29*	*<0.0001*	43.68^{a}	20.50^{b}	*4.79*	*0.006*
Ratio n-6/n-3	46.47^{a}	12.88^{b}	*5.86*	*<0.0001*	49.72^{a}	21.78^{b}	*5.7*	*0.006*
Ratio PUFA/ SFA	0.46^{a}	0.62^{b}	*0.03*	*0.022*	0.56	0.64	*0.03*	*0.24*

*Different superscripts, significant differences (P≤0.05); T = the composition in FA tend to be influence by the treatment applied (P<0.10).

***Sum* SFA = C14:0 + C16:0 + C18:0; Sum MUFA = C14:1 + C16:1 + C18:1cis-9; Sum PUFA = C18:2n-6 + C18:3n-6 + C18:3n-3 + C20:4n-6 + C22:6n-3 + CLA.

The α-linolenic FA increased significantly by addition of camelina oil whatever the type of muscle (>3.83 times in *Longissimus dorsi,* P<0.0001, respectively >2.39 times in *Semitendinosus* muscle*,* P<0.02). The level of this FA was more important (>48%) in LD muscle vs. ST muscle when camelina oil was added. A better incorporation of α linolenic FA in tissue was observed by Kouba, 2003 by using dietary linseed (the values was between 2-3%), a possible explication consisting in the higher content in this FA in linseed vs. camelina. Furthemore, in the camelina oil an important part of linolenic FA is gamma. Nuernberg, 2005, observed a better incorporation of C18:3n-3 in muscle lipids by using linseed oil (8.5% on male and 9.1% on female) and Zahan, 2010, found in Mangalitsa intramuscular fat, 1.40% C18:3n-3 FA close to our results. Contrary, Corino et al. 2003 and Nuernberg, 2005, found a

decreased level of by addition of tallow (0.60%), corn oil (0.58%) and rapeseed oil (0.80%), respectively olive oil (0.6% - 0.7%).

The increase in n-3 FA was accompanied by a corresponding decrease in arachidonic acid in the muscle. α-Linolenic acid (C18:3n-3) is the precursor fatty acid for the synthesis of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), which play a major role in the control of cardiovascular diseases (Kouba et al. 2003). Although in our trial we did not find EPA, we observed, however, DHA at a higher level in the camelina oil supplemented group in both types of muscles. The differences for DHA were significant vs. sunflower oil group ($P<0.001$). The reduction of C18:2n-6 : C18:3n-3 ratio was important (3.44 times in LD and 2.13 times in ST muscle, $P<0.0001$) as well as of n-6:n-3 ratio (3.60 times in LD muscle and 2.28 times in ST muscle). This decrease was due to the significant increase in C18:3n-3 and DHA percentage and to the decrease in C20:4n-6. The level of n-3 FA of muscle is correlated with the intake of these FA (Pearson correlation coefficient =1). The level of saturated FA was close to 40% in the both muscles but the differences were not significant between the groups ($P>0.05$).

Biochemical parameters of blood plasma

Lipid profile

The biochemical parameters of plasma are important markers to evaluate the subclinical health as a valuable tool in modern preventive medicine. Several biochemical variables are strongly influenced by the chronic disease and nutritional deficiencies (Friendship, 1984). In the present study, we noticed a significant influence of camelina oil on glucose concentration (86.95 vs. 72.90) (Table 6).

Our results also showed a decreased tendency in the level of cholesterol of pigs fed camelina oil diet compared to the pigs fed sunflower oil diet (60.12 vs. 71.16; <18%), but the differences were not significant ($P>0.05$). The high level of PUFA in the camelina oil could have an influence on the level of cholesterol. The concentration of triglycerides was also lower in the camelina oil group than in the sunflower oil group (30.10 mg/dl vs. 49.82 mg/dl; < 65%) (Table 6).

Table 6. Glucose, cholesterol and triglycerides level on blood plasma in finishing pigs

Variable	Groups*								*SEM*	*P***
	Sunflower oil				Camelina oil					
	Minim	*Maxim*	Mean	*CV %*	*Minim*	*Maxim*	Mean	*CV %*		
Glucose (mg/dl)	62.80	95.10	72.90[a]	*15.99*	79.00	99.70	86.95[b]	*8.56*	*3.43*	*0.03*
Cholesterol (mg/dl)	50.20	96.50	71.16	*25.27*	55.90	65.70	60.12	*5.98*	*3.94*	*0.17*
Triglycerides (mg/dl)	21.00	56.40	49.82	*41.00*	22.70	43.20	30.10	*23.1*	*9.19*	*0.31*

* *references of* n*ormal values*: glycaemia : glycaemia : 66.4 – 116.1 mg/dL; cholesterol: 81.4-134.1 mg/dL; triglyceride: 33-50 mg/dL. The Merk Veterinary Manual (MVM). Reference Guides, 1998.
** a,b – different letters = significant differences between groups ($P<0.05$).

Much literature data indicate that the levels of lipids and cholesterol depend on the breed of pigs, their genotype in relation to lipoproteins, sex and the type of feed given (Hebean et al., 2008, Kloareg et al., 2007, Pond et al., 1997). Over the years, numerous feeding studies corroborated the blood cholesterol predictive equations and showed that saturated fatty acids were hypercholesterolemic and that polyunsaturated fatty acids lowered blood cholesterol concentrations (Etherton, 1997).

Protein profile

Table 7 shows the protein profile in plasma of pigs fed ecologic and conventional diets; no differences were noticed between the two groups.

The total protein concentration was within the range of 7.48 g/dl to 8.82 g/dl in control group and from 7.21 g/dl to 8.08 g/dl in the camelina oil group, in both cases the value being within the physiological limits.

Table 7. Protein profile on blood plasma in finishing pigs

Variable	Groups*								*SEM*	*P***
	Sunflower oil				Camelina oil					
	Minim	*Maxim*	Mean	*CV%*	*Minim*	*Maxim*	Mean	*CV %*		
Total protein (g/ dl)	7.48	8.82	7.98	*6.94*	7.21	8.08	7.66	*4.52*	*0.13*	*0.25*
Albumin (g/ dl)	3.53	3.89	3.69	*3.97*	3.28	3.99	3.69	*3.97*	*0.05*	*0.56*
Creatinine (mg/dl)	1.70	1.86	1.80^{a}	*3.30*	1.52	1.80	1.65^{b}	*6.93*	*0.03*	*0.02*
Urea (mg / dl)	32.00	59.00	44.33^{T}	*20.8*	27.00	44.00	34.33^{T}	*19.8*	*2.69*	*0.06*

* Reference of normal value: protein: 5.8 - 8.3 g/dL; albumin: 2.3 - 4 g/dL; creatinine: 0.8 - 2.3 mg/dL; urea: 8.2 – 24.6 mg/d. The Merk Veterinary Manual (MVM) Reference Guides, 1998.
** a,b – different letters = significant differences between groups (P<0.05),
T - P=0.06 – there are a tendency to be influenced.

The data recorded for albumin were slightly lower than the normal values and for creatinin they were within the recommended limit and once again no effect of camelina oil on these two biochemical parameters was noticed. Mean urea value in our samples was higher than the limit recommended in both groups but there is a wide variability.We noticed an almost significant decrease (P=0.06) in the concentration of urea in plasma of pigs receiving camelina oil and the urea to creatinin ratio was lower in the camelina oil group (urea:creatinin = 20).

Conclusions

The dietary addition of camelina oil as sources rich in n-3 PUFA altered significantly the level of n-3 PUFA in animal tissue with consequences on human health. Although *Longissimus dorsi* muscle had a higher content of fat than *Semitendinos* muscle, n-3 PUFA incorporation occurred preferentially in *Longissimus dorsi*. The accumulation of long-chain n-3 fatty acids (DHA) was important in the camelina oil group, while these FA weren't identified in control group. The higher content of n-3 PUFA in the camelina oil influenced particularly the plasma lipid composition. The concentration of cholesterol and triglycerides was decreased probably due to the content in n-3 FA. The results obtained are very important for a better understanding of the biochemical processes in finishing pigs for estimating their physiological status and also for medical human researches.

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