EFFECT OF DIETARY N-3 POLYUNSATURATED FATTY ACIDS ON *LONGISSIMUS DORSI* AND *SEMITENDINOSUS* MUSCLE IN PIGS

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Abstract

The aim of this study was to investigate the effects of dietary linolenic acid in pigs in growing-fattening-finishing period on performances, fatty acids (FA) composition on two types of muscle (Longissimus dorsi, LD and Semitendinosus, ST) and carcass quality. Gas chromatography method was used to determine the composition in FA and cholesterol. Intramuscular fat was determined gravimetrically by Soxlet method and protein content by Kjeldhall method. Addition of Camelina oil in the E group diet determined a significant increase of C18:3n-3 FA content (P<0.0001) whatever the type of muscle. Camelina oil with a high content of n-3 FA improved nutritional value of meat favouring incorporation of C18:3n-3 in muscle and adipose tissue and its conversion into long chain FA (especially, eicosadienoic si DHA). In addition, such nutritional strategy has allowed the incorporation of (CLA) conjugated linoleic acid, all of them being beneficial for human health.

Key words: n-3 FA, camelina oil, linoleic FA:linoleic FA ratio, hybrids pigs

INTRODUCTION

Recommendations on human nutritional intake are oriented to increase fatty acids (FA) omega 3 (ω3) consummation, omega 6 (ω6) family reduce and the tendency to reduce the n-6: n-3 ratio to around 5 (ideal ratio reached for humans is 5:1, [11]). Changes in pigs feed ingredients using a high content n-3 FA may improve the nutritional value and organoleptic qualities of meat [4, 6]. Also, various growth factors influence in an important measure lipid composition of meat, first in term of quantity of fat stored and secondly as regard FA composition. By nutritional manipulating of FA in animal products obtained, we can influence the technological, sensorial and dietary characteristic of meat [2, 6]. This increased interest in controlling FA composition on animal tissue, is due to consumer awareness of health issues that may be associated with consummation of food of animal origin [9, 12]. It was also stated that the diets are deficient in ω3 and ω6:ω3 ratio (15-20:1 vs. 1:1 of that in wild animals). In the past, more 100 years ago, has been a decrease in this ratio in all European countries. During human evolution, ω 3 FA was in all consumed food (meat, wilds plants, eggs, fish, and berries). Moreover, fast dietary changes in short period of time in the last 100-150 years are evolutionary phenomena that remain. Linolenic and linoleic FA, but also their long chains derivates are important components of cell membranes of animals and plants. A diet with increased ratio n-6: n-3 are detrimental eicosadienoics metabolism and gene expression.

In this study, we proposed to investigate the effects of linolenic acid incorporation in the diet of pigs' growth-fattening-finishing periods on livestock performance, FA composition in two types of muscle (*Longissimus dorsi,* LD and *Semitendinosus,* ST*)* and carcass quality, too.

MATERIALS AND METHODS

Animals and treatment

The experiment was conducted at SC SUINPANAGRO SRL on 22 hybrid pigs (♀ Large White x Landrace by ♂ Pietrain) for a period of 92 days. We formed two

homogeneous groups of animal (control, C, 12 heads and experimental, E, 10 head). Pigs were individually weighted at the beginning of the biological test (average 33 kg), at 63 days (passing to another category) and at the end of testing (92 days). Compound feed formulation (CF) was isoenergetic and isonitrogenous for their development and using farmer's ingredients. It was collected and analysed samples of feed ingredients and subsequently FC samples. Nutritional requirements were in agreements with NRC, 1998 [13].

Addition of fat in the diet of experimental group was the Camelina oil obtained by cold pressing and characterized by a high content of polyunsaturated FA (PUFA), particularly ω3 FA family, with antioxidant included. FC used to E group named "N.C. FS OMEGA 3" has been certified by CEEX-8 LIPOSAN project. In both groups, in vitamin premix, was included vit. E on the recommended level. It was recorded daily food consumption and permanent access to water. The animals were housed under the same experimental conditions.

Fatty acids and peroxidability index

Animals at slaughter were sampling. Two samples of muscles was taking (LD and ST, different by fibre type) in order to determine the FA centesimal composition. To determine the FA we used Gras chromatography method. Perkin-Elmer-Clarus 500 is equipped with capillary

Column injection system (split ratio around 1:100) heat able column oven temperature programmed chromatography system, equipped with flame ionisation detector (FID) and capillary column stationary phase separation with high polarity (TR-Fame, 60m X 0.25mm inner diam 0.25um.thick film). The method involves obtaining FA as methyl esters and then separated them by column chromatography and their components identification using standard chromatogram. For FA extracted from muscle and fat peroxidation index (IP) was calculated by the equation reported by Hu et al, 1989, [8] namely: IP = (% dienoic x 1) + (% trienoic x 2) + (% tetraenoic x 3) + (% pentaenoic x 4) + (% hexaenoic x 5). This index estimates the concentration of bis-allylic hydrogen atoms present in PUFA and their susceptibility to peroxidation.

Carcass quality

Assessing carcass quality (% fat, thickness area and meat: fat ratio) determination were made on live animals using ultrasound device PIGLOG. After slaughtering, intramuscular fat was determined by gravimetric method (Soxlet), this method consists in organic solvent (petrol ether) extraction followed by drying at 105°C and weighing.

The cholesterol in muscle samples was determined by gas chromatography method using Gas chromatograph device Perkin Elmer Clarus -500 equipped and Elite 5 capillary column (30m, 0.32mm ID, 0.1 µm. thick films).

In muscle tissue samples we determined the protein content by Kjedhall method, too.

Statistical analyses

The results were expressed as mean value ± standard deviation (SE). Values for FA are expressed in percent (% in total fat). Data were analysed by using STAT VIEW version SAS (Anova model) at 10%, 5%, 1% and 0,001% significance level. The experimental design consists of 2 factors of influence (treatments applied and tissue).

RESULTS AND DISSCUTIONS

Performances bio productive

Bio performances are presented in table 1 (body weight, average daily gain, AVG). Treatment had no significant influence on body weight whatever physiological category ($P>0.05$). AVG in growing –fattening and in the entire experimental period was not significantly different between groups ($P>0.05$) except fattening –finishing period (E group with Camelina oil included in FC formulation, had >24% than C group; $P=0.0002$). The difference was probably due to the influence of animal not due to the diet because these were similar in terms of index of quality. On the other hand, feed conversion (kg FC /kg gain, table 2) in II period was lower in E group compared with C group. The average daily consumption of FC whatever the weight category was close between groups.

Table 1 Performances bio productive (kg)

Specification	GROUP*	
	Control	Experimental
Initial average weight	33 ± 3.80[a]	33 ± 2.96[a]
Average weight at 63 days	95 ± 7.28[a]	94 ± 4.95[a]
Final average weight	119 ± 10.88[a]	124 ± 8.16[a]
Average daily gain growing –fattening period	1.000 ± 0.08[a]	1.023 ± 0.08[a]
Average daily gain fattening-finishing period	0.840 ± 0.15[a]	1.038 ± 0.15[b]
Total average daily gain	0.950 ± 0.09[a]	1.028 ± 0.09[a.]

** Same letter –significant differences between groups (P>0.05). Different letter – significant differences (P≤0.05)*

Table 2. Average daily intake of FC (Kg) and feed conversion (kg NC /kg gain)

Specification	GROUP	
	Control	Experimental
Average daily intake of FC –growing-fattening	2.18	2.28
Average daily intake of FC fattening-finishing	3.49	3.93
Average daily intake of FC -Total period	2.58	2.76
Feed conversion – first period	2.19	2.26
Feed conversion – second period	4.15	3.83

Fatty acids and peroxidability index

a. Fatty acids in muscle tissue

Total fatty acids profile on LD and ST muscles is showed in tables 3 and 4. The addition of Camelina oil in the diet of E group determine a significant increase in the level of linolenic acid in both LD and ST muscle (P<0.001). In the LD muscle, E group, linolenic FA content is 3.93 times higher than C group. Stands a higher content of linolenic acid in LD muscle compared with ST muscle (> by a factor of 1.26). Whatever type of tissue studied, we observed accumulation of n-3 FA with long chain to a higher level than those reported by Nuernberg, 2005, [10] but differences were not significant between groups in our case. As expected, ratio C18:2n-6/C18:3n-3 was significantly reduced (P<0.0001) whatever type of muscle, it is due to the significant increase in linolenic acid, but also in beneficial FA eicosadienoic and docosahexaenoic with the highest percentage in group with Camelina oil included. Hos et al, 2003 [7] noted the influence of diet in term of this FA n-3 long chain, contrary to data reported by Enser et al., 2000 [4] reported an increase in all PUFA n-3 long chain if we have PUFA n-3 enriched diets.

Nuernberg, 2005 [10] si Hos et al, 2003 [7] also observed a significant increase of linolenic in diet with flax added. This ingredient is of the same family with Camelina and has a composition in C18:3n-3 close to Camelina. By linolenic FA was lower in ST than LD muscle but the ratio linoleic: linolenic fatty acids were lower in ST than LD muscle due probably to higher content in linolenic acid. CLA in both type of muscles also contribute to the positive impact on human health. Increase in n-3 FA content led to a reduction in arachidonic FA. At a higher level than other FA were identified unsaturated FA, mainly due to higher proportion of oleic FA (this FA is well represented in the oil ingredient, namely sunflower and soybean meal). The ratio of saturated FA did not differ significantly between groups (mainly represented by palmitic FA, P>0.05) and, in general, are relatively lower than monounsaturated FA. PUFA content in any of the tissues depend on the amount and composition of dietary fat, FA synthesis the conversion rate on the other FA and metabolites (Nuernberg, 2005) [10]. Significant differences in term f PUFA are noted in LD muscle (P=0.0001) and in ST muscle are increasing but not significant (P=0.23).

Tabel 3. FA composition (%) in *Longissimus dorsi* muscle

Fatty acids	Control	Experimental	P
14:0	1.40 ± 0.07	1.59 ± 0.21	0.83
16:0	25.59 ± 0.88	25.83 ± 0.86	0.79
18:0	13.47 ± 1.71	12.30 ± 0.38	0.20
18-1 9-11	42.32 ± 2.60	39.84 ± 1.74	0.0078
18:2 n-6	9.99 ± 1.39	10.59 ± 1.88	<0.0001
18:3 n-3	0.84 ± 0.09	4.14 ± 070	0.0005
20:4 n-6	1.39 ± 0.02	1.01 ± 0.33	0.67
Eicosadienoic	0.14 ± 0.01	0.32 ± 0.09	0.84
22-6n-3	0.21 ± 0.05	0.11 ± 0.04	0.91
CLA	0.16 ± 0.03	0.20 ± 0.02	0.96
Sum SFA	40.46 ± 2.13	39.72 ± 1.18	0.41
Sum MUFA	46.11 ± 3.29	43.41 ± 1.93	0.0038
Sum PUFA	12.73 ± 1.34	16.34 ± 2.73	0.0001
18:2n-6 / 18:3n-3	12.03 ± 2.00	2.59 ± 0.37	<0.0001
P/S ratio	0.31 ± 0.02	6.37 ± 1.19	<0.0001

Tabel 4. FA composition (%) in *Semitendinosus* muscle

Fatty acids	Control	Experimental	P
14:0	1.50 ± 0.24	1.54 ± 0.15	0.96
16:0	25.56 ± 0.98	25.85 ± 0.57	0.72
18:0	12.13 ± 1.86	12.24 ± 0.80	0.89
18-1 9-11	41.33 ± 1.28	40.08 ± 2.30	0.13
18:2 n-6	11.49 ± 1.08	10.72 ± 2.34	0.35
18:3 n-3	0.97 ± 0.12	3.28 ± 0.29	0.0064
20:4 n-6	1.75 ± 0.37	1.20 ± 0.27	0.51
Eicosadienoic	0 ± 0	0.23 ± 0.05	0.78
22-6n-3	0.24 ± 0.03	0.16 ± 0.04	0.92
CLA	0.31 ± 0.15	0.16 ± 0.07	0.85
Sum SFA	39.19 ± 0.82	39.63 ± 1.50	0.59
Sum MUFA	45.71 ± 1.90	43.81 ± 2.10	0.02
Sum PUFA	14.75 ± 1.11	15.74 ± 2.88	0.23
18:2n-6 / 18:3n-3	11.97 ± 1.05	3.25 ± 0.47	<0.0001
P/S ratio	0.38 ± 0.02	0.40 ± 0.09	0.97

b. Fatty acids in subcutaneous fat

Subcutaneous fat of pigs is less used for human consumption but its composition in this study show us a higher share in linolenic FA (>by a factor of 1.60 compared to LD muscle and 2.03 compared to ST muscle in the E group. Meanwhile, there is a reduction up to 1.91% (group E) ratio linoleic:linolenic than 3.25% in St muscle (group E) and 2.95% in LD muscle in the same group. Whatever the tissue the linoleic: linolenic ratio is reduced to a level below 5 that being a level beneficial to human health.

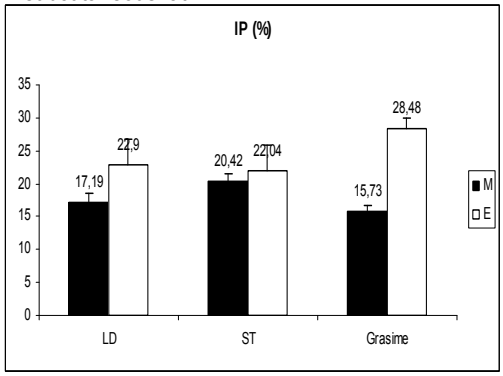
Tabel 5. FA composition (%) in subcutaneous fat

Fatty acids	Control	Experimental	P
14:0	1.56 ± 0.06	1.76 ± 0.16	0.77
16:0	23.46 ± 2.04	24.25 ± 0.66	0.26
18:0	12.15 ± 1.15	11.00 ± 0.49	0.10
18-1 9-11	46.41 ± 2.35	39.92 ± 0.32	<0.0001
18:2 n-6	11.83 ± 0.81	12.70 ± 0.43	0.08
18:3 n-3	0.18 ± 0.21	6.65 ± 0.39	<0.0001
20:4 n-6	0.12 ± 0.02	0.58 ± 0.09	0.51
Eicosadienoic	0.18 ± 0.21	0.25 ± 0.30	0.92
22-6n-3	0.13 ± 0.02	0.06 ± 0.07	0.92
CLA	0.20 ± 0.05	0.19 ± 0.03	0.99
Sum SFA	37.17 ± 2.55	37.01 ± 0.96	0.82
Sum MUFA	48.95 ± 2.40	42.38 ± 0.59	<0.0001
Sum PUFA	13.72 ± 0.85	20.43 ± 0.92	<0.0001
18:2n-6 / 18:3n-3	9.45 ± 0.99	1.91 ± 0.08	<0.0001
P/S ratio	0.37 ± 0.05	0.55 ± 0.04	0.79

c. Peroxidability index

Unsaturated fat tissue influences the shelf of the carcass resulting peroxidation phenomena. Lipid peroxidation may be a major cause of quality muscle and meat characteristics, which directly affect the favour, colour, texture, nutritional value and food safety (Buckley, 1995) [1]. Figure 1 shows the peroxidability index calculated from the composition of the FA both in LD and ST muscle. Based on the FA composition of the two muscles LD and ST, IP calculated to show greater susceptibility to peroxidation of LD muscle and fat due to increased proportion of polyunsaturated FA (group E), knowing that n-6 FA and particularly n-3 are more affected of peroxidation due to the presence of numerous positions allylic aliphatic chain [3].

Figure 1. Peroxidability index in muscle and subcutaneous fat



3.2. Carcass quality

Quantity and type of lipid stored in muscle largely depend on not only food intake but also of digestion, intestinal absorption, hepatic metabolism and lipid transport system to muscle [5]. In this study, intramuscular fat was > 1.2% at group E than C group. Thickness area and meat: fat ratio had similar values (figure 2,3).

Figure 2. Parameters of carcass quality

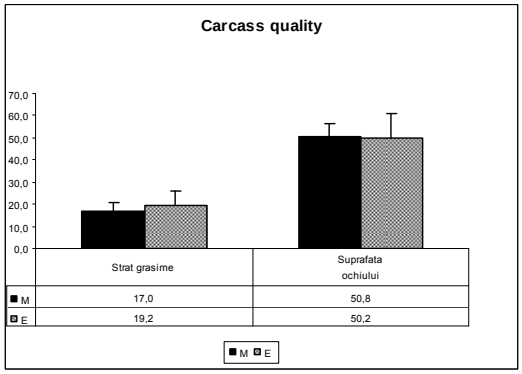
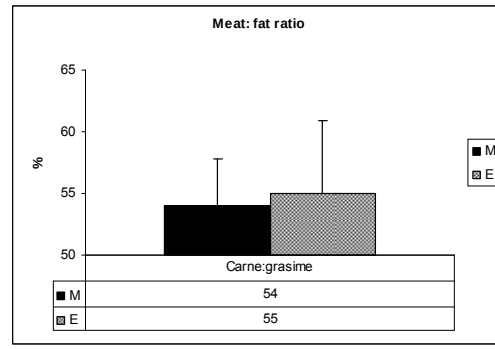


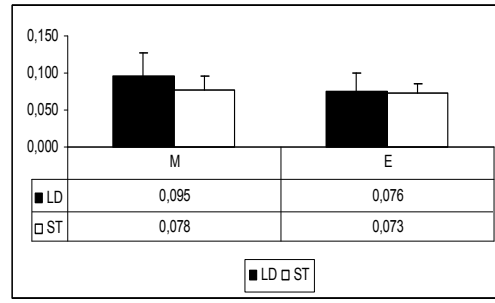
Figure 3. Meat:fat ratio*



- *determination was p*
- *erformed with ultrasound apparatus, PIGLOG, on live animals*

No significant differences were reported regarding the cholesterol level (figure 4) but it noted a reduction in group with Camelina oil included in the diet, knowing that the diet can influence cholesterol level only 15%. Meanwhile, the cholesterol content was higher in ST than LD muscle.

Figure 4. Cholesterol level in muscle tissues (%)



CONCLUSIONS

The addition of Camelina oil with a higher content of n-3 FA in pigs feeding, improve nutritional value of meat, favouring incorporation of C18:3n-3 in muscle and adipose tissue and converted to n-3 PUFA long chain (especially eicosadienoic and DHA). In addition, such a nutritional strategy has allowed the incorporation of CLA all of these FA having positive impact on health. Increased absorption of n-3 PUFA stimulates lipoperoxidation phenomena involving risks the smooth functioning of tissues (oxidant stress), affect the health value of meat for consumers. To avoid such risks was added antioxidant in Camelina oil. On the other hand, the addition of Camelina oil had an effect on the cholesterol content in tissues but not significant, given that only 15% can be influence by the diet.

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