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The relationship between blood lipid indicators and carcass traits and with the concentration of omega-3 fatty acids in the *longissimus dorsi* muscle of growing pigs

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ABSTRACT. The relationship between blood lipid indicators, subcutaneous and intramuscular fat contents and with the concentration of omega-3 fatty acids in the *musculus longissimus dorsi* (MLD) was investigated to search for biomarkers specifically associated with one of these relationships. The study was carried out on 32 gilts growing from 60 to 105 kg body weight (BW). The pigs were fed control (C) or experimental diets (L, M and H) in which 10% of metabolizable energy of diet C was replaced by 3.5% of fat mixtures that introduced in the different ratios of omega-3 fatty acids into the diets. The pigs were slaughtered at 105 kg BW and the serum concentrations of total protein (TP), triglycerides (TRIG), total cholesterol (CHOL), and high-, low-, and very low-density lipoproteins (HDL, LDL, VLDL, respectively) were determined. Backfat thickness, meat content in the carcass, and the fatty acid composition of MLD were estimated. Increased omega-3 fatty acid contents in the diet resulted in decreased concentrations of blood lipid indicators. TRIG displayed a significant correlation with meat content and backfat thickness in the carcass (r = –0.54, P < 0.01 or r = 0.43, P < 0.05). Also, a significant correlation was found between TRIG in the blood and the concentration of eicosapentaenoic and docosapentaenoic acids in the MLD (average r = –0.56, P < 0.01) and between CHOL in the blood and the concentration of linolenic acid in the MLD (r = –0.61, P < 0.01). Although the presented relationships were shown to be statistically significant, these blood lipid indicators should be viewed with caution as biomarkers specifically associated with carcass fatness.

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Introduction

Lipid content and fatty acid (FA) composition are important factors in both the nutritional quality of meat and human health. It is well known that modern human diets are characterized by a higher content of saturated fatty acids, linoleic acid and linoleic/linolenic acid ratio than recommended by Amine et al. (2002).

Therefore, in dietary recommendations for humans it is often proposed to reduce the consumption of pork to lower the risk of cardiovascular disease (Willett et al., 1995). Other authors propose improving the health value of meat by changing the fatty acid profile of pig fat depots. An effective method of achieving this, thereby modifying the human diet, is changing the diet for pigs (Nguyen et al., 2003a;

Raj et al., 2010; Kouba and Sellier, 2011; Wojtasik et al., 2012).

Among the different fatty acid groups, long-chain omega-3 polyunsaturated fatty acids (LC omega-3 PUFA) are of particular interest due to their beneficial role in human health. In monogastric animals (pigs, poultry, ostrich) and humans, linoleic acid is an essential fatty acid, so it must be supplied in the diet and can subsequently be bioconverted to longer and more unsaturated long-chain omega-6 and omega-3 PUFA. It should also be emphasized that humans and pigs can metabolize about one-third of consumed linolenic acid to eicosapentaenoic and docosapentaenoic acids, and less to docosahexaenoic acid, as well as linoleic to arachidonic acid, AA (e.g., Kloareg et al., 2007). Moreover it was found that as humans age, the activity of their enzymes (i.e. elongases, Δ5 and Δ6-desaturases) involved in the biosynthesis of docosapentaenoic and docosahexaenoic or arachidonic acids is decreased (Heird and Lapillonne, 2005). It seems that diets for pigs producing healthy meat should include long-chain omega-3 PUFA since the linolenic acid as a precursor of long-chain omega-3 PUFA cannot be considered as the only source of EPA and DHA in the diet.

Recent studies have also shown that blood lipid indicators can be used as markers to assist in the selection for meat quality and, specifically, for the deposition of lipids and fatty acids (Gao et al., 2007; Cánovas et al., 2009). The concentrations of lipoproteins, cholesterol and triglycerides in the blood serum are the results of processes occurring in lipogenic tissues of the body under the influence of genetic and dietary factors (Cánovas et al., 2009). Determining these relationships can help understand the specific linolenic acid metabolic pathways of fat.

Only a few studies have examined the relationship between the concentration of lipid indicators in the blood with the fat content and FA profile in tissues (e.g., Muñoz et al., 2012). The results of this work show only a small relationship between the concentration of blood lipid indicators and the contents of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in tissues of pigs fed diets with different FA profiles. However, the relation between lipid blood indicators and FA profile in animal tissue when omega-3 PUFA was added to diet was not investigated.

Thus, the objective of the present study was to evaluate the relationship between selected indicators of the blood serum and the health parameters of the *musculus longissimus dorsi* and/or carcass fatness in pigs fed diets supplemented with different amounts of linolenic, eicosapentaenoic, docosapentaenoic and docosahexaenoic acids.

The observed relations could be used as potential biomarkers for indicating the carcass subcutaneous fat deposition and concentration of omega-3 in the meat.

Material and methods

Animals

The study was carried out on 32 cross-breed gilts [(♂ Duroc × ♀ (♂ Danish Landrace × ♀ Polish Large White)] growing from 25 to 105 kg body weight (BW). The animals were free of genes responsible for meat defects. The origin of the pigs minimized their genetic variability as they were the offspring of one boar and eight sibling sows. The pigs were kept in individual pens (1.2 × 2.8 m, concrete floor without straw) equipped with a nipple drinker and feeder, under thermo-neutral conditions: air temperature 18–20°C, 60%–70% humidity, 0.2–0.4 m · s^{-1} of draught. The concentrations of carbon dioxide, hydrogen sulphide and ammonia were in accordance with Polish law (Regulation of the Minister of Agriculture and Rural Development, 2003).

Diet and feeding

During growth from 25 to 60 kg BW, all of the animals were fed a commercial grower diet (13.2 ME MJ ·kg^{-1} and 8.2 g · kg^{-1} ileal digestible lysine). At 60 kg BW, the pigs were allotted to 4 groups (8 gilts each) and up to 105 kg BW (by 51 days, on average) they were fed a control or experimental diet. The diets contained similar amounts of metabolizable energy (average 13.5 ME MJ · kg^{-1}) and standardized ileal digestible lysine (average 7.4 g · kg^{-1}). Standardized ileal digestible amino acids were balanced and the lysine:energy ratio (Lys g · MJ^{-1} ME) equalled 0.55 (Degussa, 2001). The components, chemical composition and nutritive value of the diets are given in Table 1.

In the experimental diets (L, M and H), approximately 10% of the metabolizable energy of diet C was replaced by 3.5% of fat mixtures that introduced the different ratios of omega-3 fatty acids into the diets. The fat mixtures contained, %: rapeseed oil 1.0, fish oil 2.0 and lard 0.5 (diet L); rapeseed oil 2.5 and linseed oil 1.0 (diet M); rapeseed oil 1.0, linseed oil 2.3 and lard 0.2 (diet H). The diets differed in the amounts of linolenic (C18:3 n-3, ALA, from 0.6 to 8.5 g · kg^{-1}), eicosapentaenoic (C20:5 n-3, EPA, from 0.00 to 0.70 g · kg^{-1}), docosapen-

Table 1. Composition and nutritive value of diets (g · kg^{-1}) fed to pigs from 60 to 105 kg body weight

Indices	Diet[1]			
	C	L	M	H
Ingredients				
barley	305	360	360	360
wheat	300	360	360	360
maize	250	100	100	100
rapeseed meal (31%)	40	40	40	40
soyabean meal (44%)	80	80	80	80
rapeseed oil	–	10	25	10
linseed oil	–	–	10	23
fish oil (cod)	–	20	–	–
lard	–	5	–	2
Premix[2]	25	25	25	25
Chemical composition				
dry matter	891	889	892	886
ash	38	41	41	41
organic matter	853	848	851	743
crude protein	158	167	172	165
fat (ether extract)	25	62	61	59
crude fibre	34	43	40	43
starch	490	453	460	450
sugar	88	83	84	87
Nutritive value				
digestible protein (determined)	131	134	133	133
lysine[3]	7.4			
methionine[3]	2.63			
threonine[3]	5.00			
tryptophan[3]	1.31			
Metabolizable energy[4], MJ · kg^{-1}	13.35	13.50	13.50	13.45
Lys/ME, g · MJ^{-1}	0.55	0.55	0.55	0.55

[1] C – control diet without oil, L – 10 g rapeseed oil, 20 g fish oil and 5 g lard, M – 25 g rapeseed oil and 10 g linseed oil, H – 10 g rapeseed oil, 23 g linseed oil and 2 g lard; [2] addition of 2.5% premix introduce to 1 kg diet: IU: vit.A 1500, vit. D_3 300, mg: Fe 60, Zn 50, Cu 30, Mn 30, I 0.30, Se 0.20, vit. E 150, vit. K_3 2.0, vit. B_1 2.0, vit. B_2 2.5, vit. B_6 2.0, vit. B_{12} 0.02, biotin 0.11, folic acid 0.6, nicotinic acid 15, calcium-D pantothenate 10, choline chloride 500; g: Ca 2.8, P 0.07, NaCl 3; essential amino acids: L-lysine-HCl 2.63, DL-methionine 0.68, L-threonine 0.98; [3] standarized ileal digestible amino acids (g · kg^{-1}) calculated according to the Degussa (2001); [4] calculated according to the GfE (2008)

Table 2. Content of fatty acids in diets (g · kg^{-1}) fed to pigs from 60 to 105 kg body weight

Fatty acids	Diet[1]			
	C	L	M	H
Total fatty acids	21.1	50.0	50.0	52.4
SFA	5.4	9.6	7.6	9.3
MUFA	6.2	16.7	17.4	15.1
PUFA	9.6	23.6	24.7	28.0
PUFA/SFA	1.8	2.5	3.3	3.0
C16:0	4.6	7.2	5.9	6.9
C16:1	0.07	0.78	0.08	0.22
C18:0	0.6	1.3	1.0	1.8
C18:1	5.6	14.0	16.7	14.0
C18:2 n-6	8.8	19.6	19.6	19.3
C18:3 n-3	0.60	2.3	5.1	8.5
C20:4 n-6	0.09	nd	nd	nd
C20:5 n-3	nd	0.70	nd	0.03
C22:5 n-3	nd	0.12	nd	0.03
C22:6 n-3	0.01	0.93	nd	0.15
PUFA n-3	0.6	4.1	5.1	8.7
PUFA n-6	8.9	19.6	19.6	19.3
C18:2 n-6/C18:3 n-3	14.6	8.5	3.8	2.3
PUFA n-6/n-3	8.9	4.8	3.8	2.2

[1] see Table 1; SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids (PUFA); nd – not determined

taenoic (C22:5 n-3, DPA, from 0.00 to 0.12 g · kg^{-1}) and docosahexaenoic (C22:6 n-3, DHA, from 0.00 to 0.93 g · kg^{-1}) acids (Table 2). The experimental diets contained 150 mg · kg^{-1} of vitamin E to protect PUFAs against autoxidation.

Pigs were fed restrictively twice a day. Daily feed allowances were adjusted weekly after BW control. The apparent digestibility of energy and chemical ingredients of feeds were determined when the pigs reached approximately 80 kg BW, using the indicator method with Cr_2O_3 after a three-day faeces collection. The metabolizable energy of the diets was calculated based on digestible nutrient components, correcting for the digestible protein, fat, starch and simple sugar contents of the organic matter according to GfE (2008).

Sample preparation

The animals were electrically stunned then slaughtered at approximately 105 kg BW. Blood was drawn into clean tubes containing heparin and centrifuged (10 min, 3500 rpm). The following biochemical indicators of blood serum were determined: total protein (TP), triglycerides (TRIG), total cholesterol (CHOL), and high-, low-, and very low-density lipoproteins (HDL, LDL, VLDL, respectively). The determinations were done on a VITROS DT 60 II System using diagnostic kits from ICN Instruments Poland Ltd.

The carcasses were chilled for 24 h at 4°C and then the entire *musculus longissimus dorsi* (MLD) was separated from the left half-carcass and weighed. The MLD was ground and a random sample of 500 g was taken and homogenized, packed into vacuum bags, frozen, and kept at –40°C until analysis of ether extract and determination of fatty acid composition.

Chemical analysis

The chemical composition of the diets, faeces and MLD was determined according to AOAC (2005) methods. Lipids of diets and MLD samples were extracted with chloroform-methanol (2:1) according to the method described by Folch et al. (1957). Fatty acid methyl esters were separated by gas chromatography on a GC-2010 AF Schimadzu gas chromatograph equipped with a 60 m capillary column (BPX70) having a 0.25 mm inner diameter and coating thickness of 0.25 mm. The operating conditions were: helium was the carrier gas, the split ratio was 1:100, injector temperature 260°C, detector temperature 260°C, the initial column temperature 140°C was held for 1 min, then increased to 200°C at a rate of 4°C · min^{-1}, then increased to 220 °C at a rate of 1°C · min^{-1}. The total run time was 36 min. Individual FA peaks were identified by comparison with a commercial standard, Supelco 37 Component FAME Mix. The concentration of fatty acids was expressed as a percentage of total FA in the investigated tissue.

Statistical analysis

Statistical analysis was performed using Statgraphics Centurion (2011) software. The effect of diets on the carcass characteristics of pigs was examined with one-way ANOVA. Differences between groups were tested using the Tukey test. Due to the close relation between the animals (litters) and the identical age of the pigs at slaughter, the influence of these factors was not included in the models. Mathematical relations between the content of linolenic acid in the diets and selected blood indicators at 105 kg BW were expressed as a curvilinear model (exponential regression equation) according to the formula:

$$Y = a \cdot X^b$$

where: Y – concentration of selected blood indicators; a – intercept; b – slope ratio; X – content of linolenic acid in the diets (g · kg^{-1}). The relations between the blood lipid indicators at 105 kg BW and carcass traits, as well as the concentrations (%) of linolenic, eicosapentaenoic, docosapentaenoic, docosahexaenoic acids, and LA/ALA or PUFA/SFA ratios in the *musculus longissimus dorsi* were expressed as Pearson's correlations.

Results

The metabolizable energy concentration and digestible protein content were similar in all of the diets (average 13.5 MJ · kg^{-1} and 133 g · kg^{-1}, respectively; Table 1). Dietary fatty acid contents (g · kg^{-1}) are shown in Table 2. In all groups of pigs, average daily feed intake, daily body weight gain, feed conversion ratio, carcass weight, meat content and backfat thickness were similar (Table 3).

Table 3. Performance and carcass characteristic of animals (n=32)

Traits	Diet[1]				SEM	*P*
	C	L	M	H		
Days of fattening	51.5	51.5	50.6	51.3	0.09	ns
Body weight, kg	104	105	104	105	0.62	ns
Feed intake, kg · day^{-1}	2.50	2.51	2.51	2.51	0.011	ns
Daily gain, g	945	972	953	931	24	ns
FCR, kg feed · kg^{-1} gain	2.65	2.58	2.63	2.70	0.010	ns
Cold carcass, kg	81.3	81.6	80.3	80.8	0.81	ns
Meat content, %	61.2	61.7	61.5	60.1	0.79	ns
Backfat thickness[2], mm	19.5	19.5	19.9	20.2	1.68	ns

[1] see Table 1; [2] backfat thickness - mean from 5 measurements (on the neck, over last thoracic vertebra and over beginning, middle and end of the muscle *gluteus medius* – on the cross); FCR – feed conversion ratio; ns – not significant

Blood lipid indicators

Pigs fed diets enriched with different fat mixtures differed in the concentration of the blood lipid indicators compared with those fed the control diet (Table 4).

Increasing the linolenic acid content in the diet lowered the concentration of total protein by 8.0% ($P < 0.049$), total triglycerides by 32.0% ($P < 0.002$), total cholesterol by 10.4% ($P < 0.05$) and HDL-cholesterol by 17.3% ($P < 0.049$) in the blood serum. There were no differences in the concentration of LDL-cholesterol, while increased intake of linolenic acid lowered the concentration of VLDL-cholesterol by 36.8% ($P < 0.001$). Supplementation of diet L with long-chain omega-3 PUFA slightly decreased the lipid indicators in blood plasma compared with diets M and H.

Table 4. Concentration of blood lipid indicator in the pigs (n=32)

Indicator[1]	Diet[2]				SEM	*P*
	C	L	M	H		
TP, g · l^{-1}	68.0^{b}	65.3^{b}	66.3^{b}	62.5^{a}	1.264	0.049
mmol · l^{-1}						
TRIG	0.41^{A}	0.27^{B}	0.31^{B}	0.28^{B}	0.023	0.002
CHOL	2.31^{b}	2.14^{a}	2.19^{a}	2.07^{a}	0.157	0.050
HDL	1.04^{b}	0.90^{a}	0.90^{a}	0.86^{a}	0.082	0.049
LDL	1.08	1.12	1.15	1.08	0.091	0.087
VLDL	0.19^{b}	0.12^{a}	0.14^{a}	0.12^{a}	0.050	0.001

[1] TP – total protein, TRIG – total triglycerides, CHOL – total cholesterol, HDL – high-density lipoprotein, LDL – low-density lipoprotein, VLDL – very low-density lipoprotein; [2] see Table 1; [a,b] means with different superscipts within a row are significantly different at $P \leq 0.05$; [A,B] means with different superscripts within a row are significantly different at $P \leq 0.01$

Table 5. Relationship ($Y = aX^b$)[1] between the content of linolenic acid (ALA) in the diets (g · kg^{-1} diet, X) and concentration of blood indicators (Y) in the pigs (n = 32)

Blood indicators[2] (Y)	a	b	Content, g · kg^{-1} diet (X)	*r*	*P*
TP, g · l^{-1}	67.126 ± 0.494	-0.029 ± 0.005	ALA	–0.97	0.001
mmol · l^{-1}					
TRIG	0.377 ± 0.006	–0.182 ± 0.015	ALA	–0.98	0.006
CHOL	2.277 ± 0.010	–0.040 ± 0.005	ALA	–0.95	0.006
HDL	0.990 ± 0.020	–0.069 ± 0.005	ALA	–0.97	0.044
LDL	1.104 ± 0.029	–0.002 ± 0.018	ALA	–0.27	0.740
VLDL	0.170 ± 0.008	–0.172 ± 0.039	ALA	–0.97	0.048

[1] Y – concentration of selected blood indicators, a – intercept, b – slope ratio, X – content of linolenic acid in the diets; [2] see Table 4

Table 6. Weight of the *musculus longissimus dorsi* (MLD, kg), concentration (%) of intramuscular fat (IMF) and SFA, MUFA, PUFA and particular fatty acids of pigs (n=32)

Traits	Diet[1]				SEM[3]
	C	L	M	H	
MLD	2.6	2.4	2.6	2.4	0.071
IMF	2.9	3.4	3.1	3.3	0.351
Fatty acids[2]					
SFA	39.3^{B}	36.4^{A}	35.4^{A}	37.5^{A}	0.480
MUFA	44.1^{B}	42.2^{A}	42.1^{A}	41.8^{A}	0.460
PUFA	11.6^{A}	13.9^{B}	14.9^{C}	15.3^{C}	0.431
PUFA/SFA	0.29^{A}	0.38^{B}	0.42^{B}	0.41^{B}	0.014
18:2 n-6	10.1	10.6	11.2	10.1	0.376
18:3 n-3	0.66^{A}	0.88^{A}	1.46^{B}	1.91^{C}	0.196
20:4 n-6	0.43^{A}	0.76^{B}	0.70^{B}	0.74^{B}	0.033
20:5 n-3	0.00^{A}	0.54^{B}	0.58^{B}	0.53^{B}	0.094
22:5 n-3	0.11^{A}	0.72^{B}	0.77^{B}	0.70^{B}	0.089
22:6 n-3	0.14^{A}	0.42^{C}	0.35^{B}	0.32^{B}	0.047
PUFA n-6	10.5	11.3	11.9	10.8	0.387
PUFA n-3	0.91^{A}	2.56^{B}	3.16^{C}	3.46^{C}	0.141
18:2 n-6/18:3 n-3	15.3^{D}	12.0^{C}	7.7^{B}	5.3^{A}	0.546
PUFA n-6/n-3	11.6^{D}	4.4^{C}	3.8^{B}	3.1^{A}	0.153

[1] see Table 1; [2] see Table 1; [3] SEM – standard error of mean; ^{A,B,C,D} means with different superscripts within a row are significantly different at $P \leq 0.01$

The relationships between linolenic acid content in the diets and the concentrations of total protein, triglycerides and cholesterol, and of HDL-, LDL- and VLDL-cholesterol in the blood serum are shown in Table 5. These relations are curvilinear and statistically significant for TP, TRIG, CHOL, HDL and VLDL. An increase the dietary ALA content by 1 g resulted in reduction of TP, TRIG, CHOL, HDL and VLDL by –0.029, –0.182, –0.040, 0.069 and –0.172 mmol · l^{-1}, respectively. The correlation coefficients for all measured indicators were similar and ranged from $r = –0.95$ for CHOL ($P < 0.001$) to $r = –0.98$ ($P < 0.006$) for TRIG. No relationships were found between the content of linolenic acid in the diets and the concentrations of LDL in the blood serum.

The fatty acid concentrations in *musculus longissimus dorsi*

The weight of the MLD did not differ among treatment groups (average 2.55 kg), but content of the intramuscular fat in the C animals was lower than in the remaining groups (2.9% vs average 3.25%, difference non-significant; Table 6).

The concentrations of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were similar in all experimental groups (average 36.4% and 42.0%, respectively), however, lower ($P < 0.01$) than in group C (39.3% and 44.1%, respectively). Concentrations of polyunsaturated fatty acids (PUFA) differed ($P < 0.01$) among groups and were the lowest in group C (11.6%), higher in group L (13.9%) and highest in groups M and H (average 15.1%). The PUFA/SFA ratio was similar in all experimental groups, however, it was higher ($P < 0.01$) than in pigs from group C (average 0.40 vs 0.29, respectively). Concentrations of linoleic acid did not differ among groups (mean 10.5%), but concentrations of linolenic acid differed significantly ($P < 0.01$) and were: 0.66% (group C), 0.88% (group L), 1.46% (group M) and 1.91% (group H). The contents of AA, EPA and DPA in the experimental groups were similar (mean 0.73%, 0.55% and 0.73%, respectively) but higher ($P < 0.01$) than in group C (mean 0.43%, 0.00% and 0.11%, respectively). The concentration of DHA was the lowest ($P < 0.01$) in group C (0.14%), higher ($P < 0.01$) in groups M and H (average 0.33%) and the highest ($P < 0.01$) in group L (0.42%). The omega-6 PUFA concentration was similar in all groups (mean 11.14%), whereas that of omega-3 PUFA differed ($P < 0.01$) among groups and equalled: 0.91% (group C), 2.56% (group L), 3.16% (group M) and 3.46% (group H). The ratio of C18:2 n-6/C18:3 n-3 differed ($P < 0.01$) and was the highest in group C (15.3), lower in group L (12.0), even lower in group M (7.7) and the lowest in group H (5.3). When the PUFA n-6/n-3 ratio was compared among groups, however, it was found that this value was 11.6, 4.4, 3.8 and 3.1 for group C, L, M and H, respectively.

Table 7. Relationship (*r*) between blood lipids indicators and carcass traits of pigs (n=32)

Blood lipid indicators[1]	Carcass traits				
	carcass weight, kg	dressing percentage, %	backfat thickness, mm^2	meat in the carcass, %	loin eye area, mm^2
TP, g · l^{-1}	0.40*	0.29	0.13	0.42*	0.11
mmol · l^{-1}					
TRIG	–0.10	0.25	0.43*	–0.54**	-0.28
CHOL	0.13	0.43*	0.17	0.15	0.09
HDL	0.25	–0.19	0.23	–0.16	0.10
LDL	–0.22	0.53**	0.05	–0.21	0.04
VLDL	0.10	0.25	0.33	–0.04	0.17

[1] see Table 4; * $P < 0.05$, ** $P < 0.01$

Relationship between blood lipid indicators and carcass traits

The concentration of lipid indicators in the blood serum displayed a low correlation with carcass traits (Table 7). The serum TP concentration was positively correlated ($P < 0.05$) with carcass weight and meat content in the carcass ($r = 0.40$ and $r = 0.42$, respectively). However, TRIG concentrations were positively related with backfat thickness ($r = 0.43$, $P < 0.05$) and negatively with meat content in the carcass ($r = –0.54$, $P < 0.01$). Also, a positive correlation was found between serum blood CHOL or LDL and dressing percentage ($r = 0.43$ or $r = 0.53$, respectively).

Relationship between blood lipid indicators and fatty acid concentrations in the *musculus longissimus dorsi*

The relationships between blood lipid indicators and the concentration of intramuscular fat and fatty acids in the *musculus longissimus dorsi* are shown in Table 8. All of the relationships between blood lipid indicators and fatty acid concentrations and PUFA/SFA ratios in the intramuscular fat were negative.

Table 8. Relationship (*r*) between blood lipids indicators and concentration (%) of IMF, ALA and LC n-3 PUFA in the *musculus longissimus dorsi* of pigs (n=32)

Blood lipid indicators[1]	*Musculus longissimus dorsi* (n=32)						
	IMF	ALA	EPA	DPA	DHA	LA/ALA	PUFA/SFA
TP, g · l^{-1}	0.14	–0.44*	–0.07	–0.07	–0.03	0.38*	–0.18
mmol · l^{-1}							
TRG,	–0.22	–0.42*	–0.57**	–0.56**	–0.42*	0.20	–0.27
CHOL	–0.03	–0.61**	–0.29	–0.30	–0.26	0.46*	–0.48*
HDL	–0.09	–0.46*	–0.30	–0.35	–0.25	0.34	–0.17
LDL	0.09	–0.43*	–0.01	–0.02	–0.09	0.32	–0.17
VLDL	–0.11	–0.45*	–0.08	–0.07	–0.06	0.25	–0.22

[1] see Table 4; IMF – intramuscular fat, ALA – linolenic acid, EPA – eicosapentaenoic acid, DPA – docosapentaenoic acid, DHA – docosahexaenoic acid; * $P < 0.05$, ** $P < 0.01$

The highest negative correlation was found between circulating total protein and concentration of linolenic acid ($r = –0.44$, $P < 0.05$). Moreover, circulating total protein showed a positive correlation with the LA/ALA ratio ($r = 0.38$, $P < 0.05$). The correlations between the level of total triglycerides in the serum and the concentration of EPA and DPA in the intramuscular fat of the *musculus longissimus dorsi* were similar and higher than with linolenic acid and DHA (mean $r = –0.57$; $P < 0.01$, vs $r = –0.42$, $P < 0.05$, respectively) and with the PUFA/SFA ratio ($r = –0.27$, $P > 0.05$). The highest correlation of total serum cholesterol concentration was with linolenic acid ($r = –0.61$, $P < 0.01$). Moreover, a positive correlation between total serum cholesterol and the ratio of linoleic/linolenic acid ($r = 0.46$, $P < 0.05$) and a negative correlation with the PUFA/SFA ratio ($r = –0.48$, $P < 0.05$) were found. A negative correlation was also found between circulating HDL, LDL and VLDL and the concentration of linolenic acid (mean $r = –0.45$, $P < 0.05$).

Discussion

According to our assumptions, replacing 10% of metabolizable energy of the basal diet by different fat mixtures did not influence the performance or carcass traits of animals due to the same energetic and nutritive values and feeding regime. Our results are similar to the earlier studies presented by Nguyen et al. (2003a) and Mitchaothai et al. (2007). Admittedly, the muscle of pigs of the control group had less intramuscular fat than the experimental pigs, but this difference was insignificant. In contrast, Nguyen et al. (2003a) and Wojtasik et al. (2012) found no differences in the intramuscular fat of the MLD in animals fed similarly as ours.

Our study is one of the few demonstrating the hypolipidemic effect of moderate doses of linolenic acid or long-chain omega-3 PUFA in the diet on lipid blood serum indicators in pigs. We observed a reduction of TP, TRIG, CHOL and HDL in pig serum caused by supplementation of diets by oil mixtures containing linseed, rapeseed and fish oil. The addition of these oils to the diet had no effect on the plasma LDL-cholesterol level, however. Our results are in agreement with other studies showing that diets enriched in linolenic acid and long-chain omega-3 PUFA caused a reduction of TP, TRIG, CHOL and HDL in the blood serum (e.g., Więcek et al., 2010). However, only a few studies have tried to determine the relationship between the intake of linolenic acid or long-chain omega-3 PUFA and

plasma lipid concentrations in pig serum. In our study we found a strong relationship between these parameters. We found that an increased intake of linolenic acid or long-chain omega-3 PUFA in the diet reduced the concentration of total triglycerides, total cholesterol and HDL-cholesterol in the blood plasma. Moreover, we found a strong curvilinear relationship between the content of linolenic acid in the diets and blood serum indicators. Other authors (e.g., Więcek et al., 2010) also found a beneficial effect of the concentration of long-chain omega-3 PUFA (from linseed) in feed on reducing the blood lipid concentration. However, these authors did not show the relations between the investigated parameters.

Our results are similar to those given by Yanovych et al. (2013). These researchers found that birds (geese) fed a diet containing fish oil had lower content of total triglycerides and cholesterol than those fed a diet containing soyabean oil. Similarly, Harris (1997) demonstrated that increasing the intake of long-chain omega-3 PUFA (mainly EPA and DHA) with food decreased the levels of total triglycerides in human blood serum. Sacks and Katan (2002) found that replacement of saturated or trans-unsaturated to monounsaturated or polyunsaturated fatty acids delivered from vegetable oils, reduced the total cholesterol and LDL cholesterol concentrations in serum. Contrary to our results, however, these authors found that increasing the amount of long-chain omega-3 PUFA (fish oil) in the diet decreased triglycerides, but had no effect on the LDL or HDL concentrations in the blood serum.

The different fat mixtures (linseed, rapeseed and fish oil) used in the diets did not influence the fat or total fatty acid contents in the *musculus longissimus dorsi*. As expected, however, they increased the concentration of long-chain omega-3 PUFA in the experimental diets and decreased SFA and MUFA. The beneficial effect of adding linseed oil, *Camelina sativa* oil, or fish oil to feed for other species (e.g., poultry) on the profile of animal tissues has also been demonstrated by other authors (Nguyen et al., 2003b; Jankowski et al., 2012; Pietras and Orczewska-Dudek, 2013). In our study, as the concentrations of ALA or LC omega-3 PUFA in the diets increased, the ratio of PUFA/SFA in the MLD increased, whereas the 18:2 n-6/18:3 n-3 and PUFA omega-6/omega-3 ratios decreased. Similar findings were presented by Duran-Montgé et al. (2010). Some researchers (Nguyen et al., 2003a; Kloareg et al., 2007; Duran-Montgé et al., 2010; Raj et al., 2010; Wojtasik et al., 2012), however, pointed to a possibility of differences between intramuscular (e.g., in MLD) and subcutaneous fat in terms of the intensity with which their EPA, DPA and DHA contents increased, even though the concentrations of linoleic and linolenic acids were significantly higher in the subcutaneous than in intramuscular fat. Tissue differences in the content of linoleic and linolenic acids can be explained by the different levels of incorporation of these fatty acids into them. Based on a regression coefficient, Nguyen et al. (2003a) found that the efficiency of linoleic acid deposition is smaller for intramuscular fat than for adipose tissue. This indicates that linoleic acid is preferentially incorporated into adipose tissue. Another reason for such a response could be a difference in the content of intramuscular fat of MLD and subcutaneous fat. The latter tissue is characterized by a higher fat content, thus linoleic and linolenic acids can be more easily incorporated.

In our study, significant correlations between blood lipid indicators and some commercial traits were found; a negative correlation between TRIG and meat content and a positive one between TRIG and backfat thickness. However, Muñoz et al. (2012) found positive correlations between HDL concentrations and ultrasonically measured backfat thickness and a negative correlation between the protein content and ultrasonically recorded thickness of the loin of pigs fed a diet in which the MUFA and PUFA contents were increased at the expense of SFA. This response did not depend on the age of the pigs. In turn, Mersmann et al. (1982) found no differences between lean and obese pigs in serum lipid contents. Other researchers (e.g., Casellas et al., 2010) reported moderate estimates for heritability of serum lipid traits, thereby confirming that these traits may be altered by selection. Some authors (Etherton and Kris-Etherton, 1980) showed that plasma TRIG, very-low-density lipoprotein, and high-density lipoprotein were highly and positively correlated with production traits in one-year-old pigs. Although the concentration of HDL in the blood plasma of pigs is related to carcass fatness, the physiological cause for the relationship of HDL with backfat thickness and IMF is unclear (Taylor et al., 1992). It is known that that HDL plays an important role in the transport of cholesterol from peripheral tissues only to the liver. While VLDL, which circulates through the blood, gives up its triglycerides to the liver and then to adipose and muscle tissues until its residues are converted to LDL (e.g., Pond and Mersmann, 1996).

Our results and the cited studies of other authors (Mersmann et al., 1982; Casellas et al., 2010; Muñoz et al., 2012) show that the relationships between blood lipid indicators and carcass fatness parameters are small. Thus, in the nearest future, blood lipid parameters cannot be markers for characteristics of the fat content of carcass tissues.

The present study demonstrates that inclusion of omega-3 fatty acids into the standard pig diet alters the fatty acid composition of muscle to a greater degree than the concentration of indicators in the blood serum. Our results show that the concentration of total protein, triglycerides and lipid indicators in the serum displayed a low correlation with intramuscular fat of the MLD. Notwithstanding, the data showed highly negative relationships between all blood lipid indicators and linolenic acid concentrations in the intramuscular fat and between TRG in the blood and EPA, DPA and DHA concentrations in the MLD. Circulating lipid indicators were, however, weakly correlated with fatness traits. Similar findings were presented by Muñoz et al. (2012).

Conclusions

In conclusion, an increase in the content of linolenic acid and long-chain omega-3 polyunsaturated fatty acids in pig diets decreases blood lipid indicators and enables the production of pork with a favourable linoleic/linolenic ratio. The regression formula established on the basis of our results makes it possible to predict the content of linolenic acid in the intramuscular fat of *musculus longissimus dorsi* based on the concentration of some lipid indicators in the blood. The concentration of lipid indicators in the blood serum displayed a highly negative correlation with the content of omega-3 fatty acids in the intramuscular fat of *musculus longissimus dorsi.* These relationships were stronger than the correlation between blood lipid indicators and carcass fatness parameters.

Although the presented relationships were shown to be statistically significant, the defined blood lipid indicators should be taken with caution as biomarkers specifically associated with carcass fatness.

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