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The effect of breed and feeding level on carcass composition, fatty acid profile and expression of genes encoding enzymes involved in fat metabolism in two muscles of pigs fed a diet enriched in n-3 fatty acids. A preliminary study

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ABSTRACT. The experiment was performed according to a 2 x 2 factorial design with breed and level of nutrition as factors affecting fat metabolism in pigs. Two groups of gilts, each comprising 4 Polish Large White (PLW) and 4 Synthetic Line 990 (L990) animals, were fed from 60 to 105 kg body weight on the experimental diet at 85% or 95% of assumed *ad libitum* intake. The diet contained 2% of linseed, 0.5% rapeseed and 0.5% fish oils as the source of n-3 fatty acids (FA). The carcass protein content was smaller and backfat thickness greater in L990 than in PLW pigs. Also the intramuscular fat content in the *biceps femoris (BF)* and *longissimus dorsi (LD*) muscles and their contents of total FA, saturated fatty acids, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were higher or tended to be higher in L990 than in PLW pigs, the differences between the breeds being more pronounced in the *BF* muscle. The n-6/n-3 PUFA ratio tended to be lower in the *LD* muscle, whereas in the *BF* muscle, it was lower in L990 than in PLW pigs. Feeding at the 85% level resulted in smaller backfat thickness and carcass fat content in animals of both breeds. Gene expression of stearoyl-CoA desaturase in both muscles was higher in L990 pigs, which could have resulted in the higher MUFA and PUFA contents in this breed. Gene expression of fatty acid-binding protein 4 and peroxisome proliferator-activated receptor were affected both by breed and feeding level only in the *BF* muscle.

Introduction

The dietary fat contents and fatty acid profiles of food of animal origin have been studied extensively since it was found that they have an important impact on human health. Particular attention has been paid to distribution and composition of fat in pork because both parameters can be modified by the feeding system, composition of dietary fats and animal breed since breeds differ in carcass fatness and intramuscular fat (IMF) contents (Raj et al., 2010). The energy source in the diet influences the profile and content of fatty acids in pig tissues, whereas the rate of fatty acid (FA) deposition depends on the target tissue and primal cut (Kloareg et al., 2007; Duran-Montgé et al., 2010). Moreover, some authors (Kapelański et al., 2010) found that increased carcass fatness parallels increased satu-

rated fatty acid (SFA) contents. It may, therefore, be expected that reduction of carcass fatness by restrictive feeding should improve the fatty acid profile. Nonetheless, according to some authors (Pascual et al., 2007), both lean and fat pigs respond to fat supplementation in a similar way, and the differences in fatty acid profiles result rather from carcass fatness and intramuscular fat content.

The ratio of n-6 to n-3 polyunsaturated fatty acids (PUFA) (n-6/n-3 PUFA) in meat from pigs fed a commercial feed is, in contrast to beef, high and above the value recommended by the World Health Organization (WHO/FAO, 2003), mainly because of the higher content of linoleic acid (C18:2 n-6, LA; Raes et al., 2004). LA and α-linolenic acid (C18:3 n-3, ALA) are precursors of long-chain (LC) n-6 PUFA and LC n-3 PUFA, respectively, which cannot be synthetized *de novo* by mammals and must be supplied with the diet. One of the ways to reduce (improve) the n-6/n-3 PUFA ratio in pork is to increase the n-3 PUFA content in pig feed. Natural sources of n-3 PUFA include linseed and linseed oil (rich in ALA), fish oil (a good source of eicosapentaenoic (C20:5 n-3, EPA) and docosahexaenoic (C22:6 n-3, DHA) acids), and rapeseed oil (a good source of LA and ALA, but also of monounsaturated fatty acids (MUFA); Duran-Montge et al., 2010; Raj et al., 2010).

In our previous papers (Raj et al., 2010; Skiba et al., 2012) we reported that a diet enriched with a mixture of linseed, rapeseed and fish oils has a greater beneficial impact on the fatty acid profile of pig tissues than diets containing single oils. This diet was, therefore, used in the present study on the effects of breed and feeding level on the fat content and fatty acid profile in the *longissimus dorsi* (*LD*) and *biceps femoris* (*BF*) muscles. We also planned to examine whether the potential differences in fat contents may be related to changes in the expression of genes encoding proteins participating in fatty acid metabolism and accumulation. Therefore, stearoyl-CoA desaturase (*SCD*), fatty acid-binding protein 4 (*FABP-4*), and peroxisome proliferator-activated receptor γ (*PPARγ*) genes expression was determined. To date, most studies (Gao et al., 2011; Yu et al., 2013) have estimated transcript levels in the *LD* muscle, whereas there is no data concerning the *BF* muscle, probably because of problems with sample collection in commercial slaughter houses and the generally greater emphasis placed on the polymorphism of the selected genes. Our planned analysis of both *LD* and *BF* muscles should contribute new information on this issue.

Our hypothesis was that pigs differing in carcass fatness and intramuscular fat content due to breed and feeding level also differ in fatty acid profile and in the expression of genes encoding proteins involved in fatty acid metabolism in muscles. This hypothesis was verified in the present preliminary study performed on a small number of pigs of the Polish Large White (PLW) and Synthetic Line 990 (L990) breeds differing in their subcutaneous fat and IMF contents (Skiba et al., 2013). It was assumed that the restriction of feeding level to 95% of *ad libitum* intake would limit the maximum feed intake, whereas restriction to 85% should also reduce carcass fatness.

The aim of the experiment was to investigate the changes in fatty acid profiles and transcription levels of genes connected with fat metabolism in pigs of both breeds fed restrictively with a diet enriched in n-3 PUFA.

Material and methods

The experimental procedures used throughout this study were performed in accordance with national/local ethical guidelines and approved by the III Local Ethics Committee on Animal Experimentation of the Warsaw University of Life Sciences – SGGW, Poland.

Animals and diet

The experiment was carried out on 8 Polish Large White (PLW) and 8 Synthetic Line 990 (L990) gilts. Within each breed the animals were the offspring of one boar and half-sibling sows and were free of genes responsible for poor meat quality. The pigs were kept individually on a concrete floor without straw in pens (2.6 m^2) equipped with nipple drinkers.

From 25 to 60 kg body weight (BW), all animals were fed a standard diet (13.2 ME MJ · kg^{-1} and 8.2 g · kg^{-1} standardized ileal digestible lysine). At 60 kg BW, the pigs of each breed were divided into two groups (n = 4) and fed the experimental diet at 85% and 95% of the *ad libitum* intake recorded for each breed in earlier studies (unpublished data) until they reached 105 kg BW (Table 1). The rations were increased weakly and offered in two daily meals. In the experimental diet, 9% of metabolizable energy was provided by 2% linseed oil, 0.5% rapeseed oil, and 0.5% fish oil (Table 1). The content of vitamin E (150 mg · kg^{-1} diet) was sufficient to protect PUFA against autoxidation.

Sample collection

At 105 kg BW pigs were deprived of feed for 16 h and slaughtered after electrical stunning at the

Table 1. Ingredients, chemical composition and nutritive value of diet, g · kg^{-1}

Indices	Diet
Ingredients, g · kg^{-1}	
barley	365
wheat	360
maize	100
rapeseed meal	40
soyabean meal	80
rapeseed oil	5
linseed oil	20
fish oil (cod liver oil)	5
Premix[1]	25
Chemical composition, g · kg^{-1}	
dry matter	886
ash	41
organic matter	845
crude protein	165
ether extract	59
starch	450
sugar	87
crude fibre	43
Nutritive value	
digestible crude protein, g · kg^{-1}	146
metabolizable energy, MJ · kg^{-1}	13.4
Lys standardized/ME, g · MJ^{-1}	0.55

[1]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, J 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, and essential amino acids: g: lysine 2.63, methionine 0.68, threonine 0.98

experimental slaughter house of the Institute. Immediately after slaughter (within 5 min), 20 g samples of the *LD* and *BF* muscles from the right half-carcass were collected, frozen in liquid nitrogen, and kept at –80°C until RNA isolation and further analysis of gene expression. The carcasses were weighed and chilled for 24 h at 4°C. The backfat thickness was measured on the right half-carcass along the carcass dividing line between the last thoracic and the first lumbar vertebra. The entire *LD* and *BF* muscles were excised from the left half-carcass, weighed and ground. The carcasses were then dissected into edible parts comprising meat and fat, and inedible skin and bones. Meat and fat were weighed and ground separately. Inedible parts were weighed and autoclaved for 8 h at 1.2 atm and 130°C. Samples of approximately 500 g of the *LD* and *BF* muscles, and edible and inedible parts were taken, homogenized, packed into foil bags, frozen, and kept at –20°C until analysis for protein and ether extract contents in all samples, and fatty acid composition in muscles.

Chemical analysis

Protein and ether extract content in the edible and inedible parts and fat (IMF) content in the *LD* and *BF* muscles were determined according to AOAC methods (2005).

Lipids for fatty acid analysis in the diet and in the *LD* and *BF* muscles were extracted with chloroform-methanol (2:1) according to the method of Folch et al. (1957). Fatty acid methyl esters were separated by gas chromatography on a GC-2010AF Shimadzu gas chromatograph (SHIMADZU Europa GmbH, Duisburg, Germany), equipped with a capillary column BPX70 (length 60 m, internal diameter 0.25 mm, film thickness 0.25 μm). Operating conditions were: carrier gas, helium; split ratio, 1:100; injector and detector temperature, 260°C; the initial column temperature of 140°C was held for 1 min, then increased to 200°C at a rate of 4°C · min^{-1}, and to 220°C at a rate of 1°C · min^{-1}. Individual fatty acid peaks were identified in comparison with the commercial standard, Supelco 37 Component FAME Mix (SUPELCO, Bellefonte, USA). The total content of fatty acids was calculated as 90% of ether extract (Kratz, 2003). The concentration of fatty acids was expressed in grams per 100 g of tissue, as this takes into account the fat content in the muscles.

Assay of relative mRNA expression

Total RNA was isolated from the muscles using TRIsure Reagent (Bioline Ltd., London, United Kingdom) according to the manufacturer's instructions. The amount and purity of total RNA were determined spectrophotometrically at 260 and 280 nm with the use of a NanoDrop 1000 instrument (Thermo Fisher Scientific Inc., Waltham, USA). RNA integrity was checked by 1% agarose gel electrophoresis. The synthesis of cDNA was performed using a DyNAmo^{TM} cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham, USA) according to the manufacturer's instructions. Nine hundred nanograms of total RNA was used as the starting material for reversed transcription in a reaction volume of 20 μl.

Real-time quantitative PCR assay was carried out employing the ready-to-use solution 5x HOT FIREPol® EvaGreen®qPCR Mix Plus (Solis BioDyne, Tartu, Estonia) and HPLC-grade oligonucleotide primers purchased from Genomed (Poland). Specific primers for determining the expression of a housekeeping gene and the genes of interest were designed using Primer 3 software (Table 2). Each PCR reaction contained 4 μl PCR Master Mix (5x),13.4 μl RNase-free water, 0.6 μl primers

Table 2. All genes analysed by Real-Time RT-PCR with full names, abbreviations, amplicons sizes, and primers characteristics

GeneBank Acc. No.	Gene	Amplicon Size [bp]	Primer: Forward/ Reverse	Exon	Sequence 5'→3'
NM_001206359.1	*GAPDH*	136	F	4/5	GACCAGGTTGTGTCCTGTGA
			R	6	CCCTGTTGCTGTAGCCAAAT
NM_213781.1	*SCD*	140	F	2/3	TCTGGGCGTTTGCCTACTAT
			R	3/4	ATTCTGGAATGCCATCGTGT
NM_001002817.1	*FABP4*	147	F	2/3	CAGGAAAGTCAAGAGCACCA
			R	4	CTGGTAGCCGTGACACCTTT
NM_214379.1	*PPARG*	149	F	3/4	TGAAGGATGCAAGGGTTTCT
			R	4	TATGAGACATCCCCACAGCA

GAPDH – glyceraldehyde-3-phosphate dehydrogenase; *SCD* – stearoyl-CoA desaturase; *FABP4* – fatty acid binding protein 4; *PPARG* – peroxisome proliferator-activated receptor gamma

(0.3 µl each with a working concentration of 150 nM) and 2 µl cDNA template. The reactions were run on a Rotor-Gene Q instrument (Qiagen, Dusseldorf, Germany) using the following protocol: 95°C for 15 min to activate the HOT FIREPol® DNA Polymerase, followed by 35 cycles of 94°C for 5 sec for denaturation, 59°C for 20 sec for annealing, and 72°C for 10 sec for extension. After the cycles, a final melting curve analysis with continuous fluorescence measurements was performed to confirm the specificity of the amplification.

All data were analysed by Rotor-Gene Q Series Software v.2.0.3 (Qiagen, Dusseldorf, Germany). Relative gene expression was determined using the Relative Expression Software Tool 2008, first published by Pfaffl (2001) and based on the PCR efficiency correction algorithm published previously by the same author (Pfaffl et al., 2002). To compensate for variation in cDNA concentrations and PCR efficiency between tubes, an endogenous control gene (glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*) was amplified in each sample and used for normalization.

The results are presented as the relative gene expression of the target gene vs housekeeping gene (*GAPDH*) using the group of PLW pigs fed at 95% of *ad libitum* intake calculated as 100%.

Statistical analysis

Statistical analysis was performed using Statgraphics Centurion (version 16.1.18, 2011) software (StatPoint Technologies Inc., Warrenton, USA). The effects of dietary restrictions and breed on the carcass characteristics of pigs, fatty acid profile and gene expression in the examined muscles were analysed using two-way analysis of variance (ANOVA) with a model that included the fixed effects of feeding level, breed and the feeding level × breed interaction. The significance of differences between pair-wise combinations of the least squares means was tested. The ANOVA analysis was performed after checking its two assumptions, normality (Shapiro-Wilk test) and equality of variances (Levene test).

Results and discussion

Concentration of fatty acids in the diet

The fatty acid concentration (%) in the diet is presented in Table 3. Due to the use of a mixture of fats rich in n-3 PUFA, the PUFA/SFA and LA/ALA ratios were reduced compared with the standard diet used in our previous study (Raj et al., 2010; average 3.14 and 2.30 vs 1.78 and 14.70, respectively). The ratio of LA/ALA in the basal diet without additional oils can even exceed 18 (Kloareg et al., 2005), whereas when the diet is supplemented with approximately 10% fat, this ratio depends on the source

Table 3. Concentration (%) and profile of fatty acids in the experimental diet

Fatty acids	Diet
SFA	16.61
C16:0	12.36
C18:0	2.83
MUFA	29.54
C16:1 n-7	0.92
C18:1 n-9	24.00
PUFA	52.15
n-6 PUFA	35.13
C18:2 n-6, LA	35.00
n-3 PUFA	16.98
C18:3 n-3, ALA	15.20
C20:5 n-3, EPA	0.69
C22:6 n-3, DHA	0.96
PUFA/SFA	3.14
n-6/n-3 PUFA	2.07
C18:2 n-6/C18:3 n-3 (LA/ALA)	2.30

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; n-6 PUFA – n-6 polyunsaturated fatty acids; n-3 PUFA – n-3 polyunsaturated fatty acids; PUFA/SFA – the ratio of total polyunsaturated fatty acids (PUFA) to total saturated fatty acids (SFA); n-6/n-3 PUFA – the ratio of n-6 to n-3 polyunsaturated fatty acids

of fat and is about 7.47, 16.67, 58.17, and 0.56 for tallow, high-oleic sunflower oil, sunflower oil and linseed oil supplementation, respectively (Realini et al., 2010).

Growth, performance and carcass characteristics

The feed conversion ratio was not affected by either breed or feeding level, but the daily weight gain of pigs fed larger daily allowances was on average 150 g greater than that of animals restricted to 85% of the *ad libitum* feed intake (1136 vs 985 g; respectively, $P < 0.01$).

Selected parameters of carcass and muscle composition are presented in Table 4. Carcass weight was not affected by breed (average 79.7 kg), however *BF* muscle weight was greater ($P = 0.075$) in PLW than in L990 pigs (1.22 vs 1.07 kg). The protein content in the whole carcass was higher ($P < 0.01$) in PLW than in L990 pigs (average 177 vs 171 g · kg^{-1}). The effect of breed on the fat content in the whole carcass was not significant, probably due to the small number of animals and large variability of this feature, whereas backfat thickness ($P < 0.05$) and IMF content ($P < 0.01$) in the *BF* muscle were greater, and IMF in the *LD* muscle tended to be greater ($P = 0.099$) in L990 than in PLW pigs. Our results indicate that pigs of these two breeds differ in backfat thickness and muscle fat contents.

Our results are similar to those presented by Burkett et al. (2009) and Szydłowski et al. (2011). The considerably smaller backfat thickness and IMF content in PLW pigs is the result of the selection towards reduction of backfat and a leaner carcass. The lower IMF content in both muscles of pigs with leaner carcasses is in agreement with data presented by Gerbens (2004), but not with the report of Czarniecka-Skubina et al. (2007), who claimed that pigs of different breeds may have similar backfat thickness and different IMF contents.

Feeding level did not affect carcass and muscle weights or carcass protein content. The fat content in the whole carcass tended to be smaller ($P = 0.077$) and backfat thickness ($P < 0.05$), mass ($P < 0.01$) and content of backfat ($P < 0.01$) in the carcass were significantly smaller in pigs fed at 85% than 95% of *ad libitum* intake. In contrast, the fat content in both muscles was not affected by greater feeding restriction. The meat content in the carcass tended to be higher ($P = 0.108$) in pigs fed at 85% than 95% of *ad libitum* intake.

Fatty acid composition of *longissimus dorsi* and *biceps femoris* muscles

The contents of total FA, SFA, MUFA and PUFA, as well as the contents of selected acids within each group in the *BF* muscle, are presented in Table 5. The breed of pig had significant effects on the contents of all fatty acids, which were approximately two-fold greater in L990 than in PLW pigs. This effect is in line with breed effect on IMF content.

The SFA content was similar to that of MUFA and was about two-fold greater than of PUFA. The PUFA/SFA ratio was the only parameter that was not significantly affected by breed of pigs, showing only a tendency towards higher values in PLW pigs in comparison with L990 pigs ($P = 0.107$). The n-6/n-3 PUFA ratio was significantly higher ($P < 0.01$) in PLW than in L990 animals (4.73 vs 3.89 in PLW vs L990 pigs, respectively).

Table 4. Characteristic of carcass, *biceps femoris* (*BF*) and *longissimus dorsi* (*LD*) muscles

Indices	Breed				SEM	Significance		
	PLW		L990			feeding level	breed	feeding level x breed
	% of *ad libitum* intake							
	95	85	95	85				
Cold carcass weight, kg	79.3	79.6	79.5	80.5	0.975	NS	NS	NS
Protein content in carcass, g · kg^{-1}	174	179	172	169	0.200	NS	0.004	NS
Fat (extract ether) content in carcass, g · kg^{-1}	211	201	231	206	0.090	0.077	NS	NS
BF muscle weight, kg	1.23	1.21	1.05	1.09	68.358	NS	0.075	NS
Fat (IMF) content in *BF* muscle, g · kg^{-1}	7.4	7.1	16.8	15.0	0.213	NS	0.001	NS
LD muscle weight, kg	2.51	2.36	2.38	2.42	83.077	NS	NS	NS
Fat (IMF) content in *LD* muscle, g · kg^{-1}	6.2	6.3	12.1	11.0	0.410	NS	0.099	NS
Backfat thickness, mm	21.7	16.8	25.7	22.8	2.126	0.034	0.015	NS
Mass of the backfat, kg	11.7	9.5	11.9	11.0	0.534	0.010	NS	NS
Backfat content in carcass, %	14.7	12.1	15.2	13.3	0.624	0.004	NS	NS
Meat content in carcass, %	59.6	61.9	58.6	59.7	1.011	0.108	NS	NS

PLW – Polish Large White; L990 – Synthetic Line 990; IMF – intramuscular fat; NS – not significant

Table 5. Content of total fatty acids, SFA, MUFA, PUFA and particular fatty acids in the *biceps femoris* (*BF*) muscle, g· 100 g^{-1} of tissue

Indices	Breed[1]				SEM	Significance		
	PLW		L990			feeding level	breed	feeding level x breed
	% of *ad libitum* intake							
	95	85	95	85				
Total fatty acids	0.68	0.63	1.51	1.35	0.120	NS	0.001	NS
SFA	0.25	0.23	0.57	0.50	0.044	NS	0.001	NS
C16:0	0.144	0.134	0.338	0.283	0.026	NS	0.001	NS
C18:0	0.083	0.082	0.194	0.173	0.015	NS	0.001	NS
MUFA	0.29	0.27	0.68	0.57	0.086	NS	0.001	NS
C16:1 n-7	0.014	0.015	0.042	0.033	0.037	NS	0.001	NS
C18:1 n-9	0.261	0.231	0.595	0.511	0.044	NS	0.001	NS
PUFA	0.14	0.13	0.26	0.27	0.036	NS	0.001	NS
C18:3 n-3 (ALA)	0.018	0.016	0.036	0.036	0.003	NS	0.001	NS
LC n-3 PUFA	0.008	0.009	0.015	0.021	0.002	0.081	0.001	NS
n-6 PUFA	0.118	0.108	0.206	0.214	0.023	NS	0.002	NS
n-3 PUFA	0.023	0.025	0.052	0.056	0.004	NS	0.001	NS
n-6/n-3 PUFA	5.13	4.32	3.96	3.82	0.223	NS	0.004	NS
PUFA/SFA	0.56	0.57	0.46	0.54	0.046	NS	0.107	NS

[1] see Table 4; LC n-3 PUFA – long-chain n-3 polyunsaturated fatty acids; n-6/n-3 PUFA – the ratio of n-6 to n-3 polyunsaturated fatty acids; PUFA/SFA – the ratio of total polyunsaturated fatty acids (PUFA) to total saturated fatty acids (SFA); NS – not significant

Feeding level did not affect the contents of the analysed fatty acids, except that of long-chain (LC) n-3 PUFA, which tended to be higher (P = 0.081) in more restricted pigs (0.015 vs 0.012 g· 100 g^{-1} in pigs fed at the 85% vs 95% feeding level, respectively), the interaction between breed and feeding level has not been, however, confirmed.

The contents of total FA, SFA, MUFA and PUFA, and particular FA in the *LD* muscle are shown in Table 6. The total fatty acid content and that of all of the main groups in this muscle were lower than in the *BF* muscle, in keeping with its lower fat content. The greatest difference between the muscles was in the content of PUFA, including LA and ALA, which in the *LD* muscle were present in far smaller amounts than in the *BF* muscle. The effect of breed on the contents of all fatty acids in the *LD* muscle was either significant or tended to be significant, but was less evident than in the *BF* muscle. The PUFA/SFA ratio was the only parameter not affected by breed. The ratio of n-6/n-3 PUFA tended to be higher (P = 0.058) in PLW than in L990 pigs (4.92 vs 4.04, respectively), the interaction between breed and feeding level has not been shown, however, to be statistically significant. Feeding level did not influence fatty acid contents or proportions.

The quantitative differences in the contents of all analysed FA between the breeds are related mainly

Table 6. Content of total fatty acids, SFA, MUFA, PUFA and particular fatty acids in the *longissimus dorsi* (*LD*) muscle, g· 100 g^{-1} of tissue

Indices	Breed[1]				SEM	Significance		
	PLW		L990			feeding level	breed	feeding level x breed
	% of *ad libitum* intake							
	95	85	95	85				
Total fatty acids	0.56	0.56	1.09	1.02	0.230	NS	0.053	NS
SFA	0.22	0.22	0.45	0.41	0.095	NS	0.048	NS
C16:0	0.128	0.129	0.262	0.232	0.055	NS	0.053	NS
C18:0	0.073	0.078	0.158	0.147	0.035	NS	0.046	NS
MUFA	0.25	0.25	0.48	0.43	0.102	NS	0.069	NS
C16:1 n-7	0.015	0.014	0.032	0.027	0.007	NS	0.037	NS
C18:1 n-9	0.208	0.206	0.394	0.357	0.086	NS	0.072	NS
PUFA	0.09	0.09	0.15	0.17	0.030	NS	0.036	NS
C18:3 n-3 (ALA)	0.011	0.011	0.024	0.022	0.005	NS	0.030	NS
LC n-3 PUFA[2]	0.004	0.005	0.006	0.010	0.002	NS	0.075	NS
n-6 PUFA	0.074	0.073	0.116	0.139	0.023	NS	0.040	NS
n-3 PUFA	0.014	0.016	0.031	0.032	0.007	NS	0.033	NS
n-6/n-3 PUFA[3]	5.28	4.56	3.74	4.34	0.483	NS	0.058	NS
PUFA/SFA[4]	0.41	0.41	0.33	0.41	0.034	NS	NS	NS

[1] see Table 4; [2,3,4] see Table 5; NS – not significant

to the difference in their IMF content, which was approximately two-fold greater in L990 than in PLW pigs, thus giving their meat a higher dietary energy value. In contrast, the PUFA/SFA ratios and, in particular that of n-6/n-3 PUFA, are important parameters indicative of the nutritional value of pork fat. The higher n-6/n-3 PUFA ratio in both muscles of PLW than L990 pigs indicates their lower nutritional value, particularly evident when the pigs were fed at the higher feeding level. However, the obtained values for the PUFA/SFA and n-6/n-3 PUFA ratios in the *LD* and *BF* muscles of both breeds fed at 85% of *ad libitum* intake, and in the *BF* muscle of L990 pigs fed at the 95% feeding level, were in accordance with WHO recommendations (WHO/FAO, 2003) for meat with health-promoting properties (the values should be above 0.4 and below 5, respectively for the PUFA/SFA and n-6/n-3 PUFA ratios). Similar results in the *LD* and *semimembranosus* muscles were obtained by Guillevic et al. (2009) when they fed pigs a diet with linseed, whereas when pigs consumed a diet with sunflower oil, the value of the n-6/n-3 ratio in both muscles was much higher than recommended by the WHO (average 16.7 vs below 5).

The effect of breed on fat and fatty acid contents found in the present study is in agreement with our previous results (Raj et al., 2010; Skiba et al., 2012) and with the conclusions of other authors (Burkett et al., 2009). Our data also support the results of Kloareg et al. (2007) and Skiba et al. (2012), who found that as the fat content in the tissues increases, so does the amount of deposited total fatty acids. Our results concerning the PUFA/SFA ratio do not seem to support a generally negative effect of carcass fatness on the dietary fat value (as related to the contents of these acids), as claimed by Kapelański et al. (2010). In our study, the PUFA/SFA ratio in the muscles was practically unaffected by a considerable difference of the intramuscular fat content between the breeds, whereas in Kapelański et al. (2010), the SFA contents were positively, but those of PUFA, negatively correlated with backfat thickness, weight of subcutaneous fat, total fat and IMF content in the *longissimus lumborum* muscle.

In our study, the apparent interactive effects of feeding level and breed were not statistically confirmed, probably due to the small number of animals per treatment, thus they are only speculative. They seem, however, worth some consideration. The different response of the breeds to feeding level in terms of the n-6/n-3 PUFA ratio lies in the reduction of this ratio in both muscles in more restrictively fed PLW pigs and in the far smaller reduction (in *biceps femoris*) or even increase (in *longissimus dorsi*) of this ratio in L990 animals. These potential interactive effects should be studied on a greater number of animals representing different breeds, as they may be important for developing feeding strategies aimed at production of pork having an improved nutritional value.

Expression of genes encoding enzymes connected with fat metabolism

The relative expression of the stearoyl-CoA desaturase (*SCD*), fatty acid binding protein 4 (*FABP4*), and peroxisome proliferator activated receptor gamma (*PPARG*) genes, presented as a percent of respective values in PLW pigs fed at the 95% level, are shown in Table 7. In the *BF* muscle, expression of all of these genes differed between the breeds, the expression of *SCD* and *FABP4* being greater, and that of *PPARG*, lower in L990 than in PLW pigs. Feeding level significantly affected expression of *FABP4* and *PPARG*, which were lower at the lower feeding level. The response of genes in the *LD* muscle differed from that in the *BF* muscle, since only relative expression of *SCD* was affected by breed and also by interaction of breed and feeding level (Figure 1). The relative *SCD* value was far greater in L990 than in PLW pigs, but only in animals fed at the lower feeding level, whereas it was very similar when pigs were fed at 95% of *ad libitum* intake.

The differences in transcription levels can be helpful in explaining the variation in fat deposition between the animals. Comparing Lantang and Landrace pigs differing in fat content, Yu et al. (2013) identified differences in the expression of genes encoding factors taking part either in adipocyte differentiation and function (peroxisome proliferator activated receptor gamma coactivator 1, adiponectin, Kruppel-like factor 5) or fatty acid uptake and transport (lipoprotein lipase, *FABP4*, apolipoprotein O-like). In our experiment we chose a gene encoding a transcriptional factor controlling adipogenesis and thus the amount of adipocytes — *PPARγ* — and gene encoding a protein involved in fatty acid transport and a later marker of adipogenesis — *FABP-4*.

We found that the *PPARG* expression level was higher in the leaner PLW breed animals, however, only in the *BF* muscle. This finding is not in agreement with the results of McNeel et al. (2000), who stated that *PPARG* gene expression in subcutaneous adipose tissue of genetically obese crossbreed (Duroc x Yorkshire) pigs was slightly higher than in genetically lean animals. The significantly lower expression of the *FABP4* gene in the *BF* muscle in pigs fed at the lower level of feed intake, found in our

Table 7. Relative gene expression in *biceps femoris* (*BF*) and *longissimus dorsi* (*LD*) muscles

Muscle	Inidces[2]	Breed[1]				Significance		
		PLW		L990		feeding level	breed	feeding level x breed
		% of *ad libitum* intake						
		95	85	95	85			
BF	*SCD*	100.00 ± 26.46	92.23 ± 36.91	175.76 ± 15.83	116.65 ± 33.80	NS	0.03	NS
	FABP4	100.00 ± 9.37	81.64 ± 9.88	134.25 ± 13.75	97.16 ± 17.75	0.02	0.04	NS
	PPARG	100.00 ± 8.87	73.54 ± 19.98	75.62 ± 6.65	65.43 ± 3.57	0.01	0.02	NS
LD	*SCD*	100.00 ± 26.50	62.95 ± 33.68	113.92 ± 6.11	164.40 ± 21.82	NS	0.01	0.02
	FABP4	100.00 ± 10.63	71.59 ± 40.62	73.82 ± 38.40	69.52 ± 7.35	NS	NS	NS
	PPARG	100.00 ± 30.70	56.13 ± 18.18	68.48 ± 29.54	67.64 ± 19.32	NS	NS	NS

[1] see Table 4; [2] see Table 2

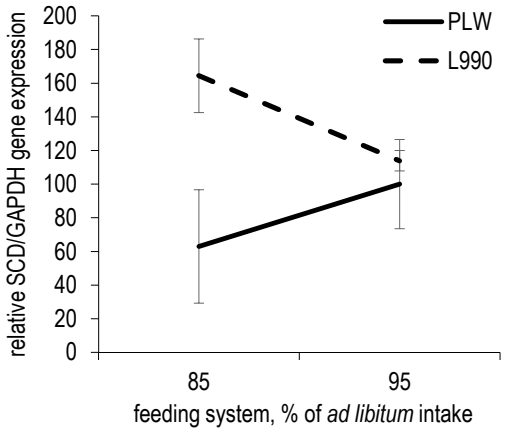

Figure 1. Relative gene expression of SCD in *longissimus dorsi* muscle. Interaction between breed and feeding system ($P < 0.05$); SCD – stearoyl-CoA desaturase; GAPDH – glyceraldehyde-3-phosphate dehyhdrogenase; PLW – Polish Large White; L990 – Synthetic Line 990

study, is not in line with the increased expression of this gene in pigs fed at the 50% restriction level reported by McNeel et al. (2000). Otherwise, our results on greater *FABP4* expression in the *BF* muscle of more fatty L990 pigs are partly in agreement with results of Damon et al. (2006), who found that in the *LD* muscle of crossbreed (Large White x Duroc) barrows, the FABP-4 protein content was two-fold greater in pigs with a high IMF content than those with a low content. These authors also found positive correlations between the FABP-4 protein level and adipocyte number and lipid content. FABP-4 is a protein examined extensively because of a positive association between IMF content and *FABP4* gene polymorphism (Gerbens et al., 1998). According to Damon et al. (2006) it is, however, possible that only pure Duroc or crossbred Duroc pigs have the correct allele in segregation. Our results are consistent with this assumption since Line 990 was established with the participation of six breeds, including Duroc. However, the greater expression of the *FABP4* gene and lower of *PPARG* in the *BF* muscle of L990 pigs, found in our study, may suggest that in L990 pigs with a final BW of 105 kg (about 27 weeks old), the increased fat content in the *BF* muscle is caused mainly by fat uptake, but with a lower intensity of adipogenesis. This interpretation is consistent with the data reviewed by Katsumata (2011) showing that the number of adipocytes in porcine muscle increased until pigs reached 24 weeks of age, or even only until 10 weeks of age.

Besides IMF content, breed also influenced the fatty acid profile. This could be connected with changes in the level of *SCD* mRNA, the first enzyme converting SFA to MUFA. Moreover, C2C12 myoblasts with overexpression of *SCD* are also characterised by increased amounts of such PUFA as: LA, ALA, arachidonic acid (C20:4 n-6) and EPA (Yu et al., 2013). In our experiment, *SCD* gene expression in *LD* and *BF* muscles was greater in L990 pigs, which also had higher MUFA and PUFA contents in these muscles. To date, differences between breeds in *SCD* gene expression have not been widely examined, and all available publications are strictly connected with the *LD* muscle. Our results are in agreement with those of Yu et al. (2013) who found that the greater SCD gene expression in the *LD* muscle of Lantang than in Landrace pigs was accompanied by higher IMF, MUFA and PUFA contents in this muscle. Opposite results were obtained by Gao et al. (2011), who reported lower *SCD* expression in Northeastern Indigenous pigs having a higher IMF content, but the animals were slaughtered on day 150 regardless of their final BW. The results reported by Wu et al. (2013) seem to indicate that *SCD* expression may be affected by the age of animals. The authors found that *SCD* expression in the *LD* muscle of Jinhua pigs that grow faster and have a higher IMF content was greater in 30-day-old pigs and smaller in animals aged 150 days. However, Bessa et al. (2013) found that SCD protein expres-

sion is associated with regulation of fat deposition only in breeds with a genetic predisposition to a low IMF content, like Large White x Landrace.

In our study, the more restricted feeding level caused higher expression of *SCD*, but only in L990 pigs and only in the *LD* muscle. The interaction of breed and feeding level can be related to the metabolic functions of SCD, which is also involved in lipid oxidation, thermogenesis and insulin sensitivity. These functions may be particularly emphasized in breeds with a higher IMF content subjected to dietary intervention.

The effects of experimental factors on gene expression in *LD* and *BF* muscles were not uniform since only *SCD* gene expression was influenced in both muscles by breed and not by feeding system. However, an interaction between breed and feeding effects on *SCD* was found in *LD* but not in *BF* muscle. On the other hand, genes involved in adipogenesis and fatty acid transport, i.e., *PPARG* and *FABP4*, were modified by both experimental factors only in the *BF* muscle. The different response of the two muscles can be referred to their diverse biochemical and physiological parameters. The *LD* muscle is classified as a white muscle having low IMF and high glycogen contents, lower activity of oxidative and higher of glycolytic enzymes, while the *BF* muscle is classified as red muscle and has a higher IMF content and lower content of glycogen, higher activity of oxidases and lower activity of glycolytic enzymes. Our results show the diversity between muscles also on the transcriptional level and their different response to nutrition in various breeds. This finding points to the importance of analysing both muscles and not only the *LD* muscle, which up to now has been examined in more detail and more frequently.

Conclusions

From this preliminary study we can conclude that reduction of the feeding level to 85% of *ad libitum* intake in Synthetic line L990 (L990) and Polish Large White (PLW) pigs reduces backfat thickness and carcass fat, but not intramuscular fat contents. The last is affected by breed and is greater in L990 than in PLW pigs. The n-6/n-3 PUFA ratio in the *biceps femoris* and *longissimus dorsi* muscles is not affected by the level of nutrition, but is influenced by breed and the ratio is higher in PLW pigs. The expression of genes involved in lipid metabolism is more sensitive to nutritional and genetic factors in the *biceps femoris* muscle than in the *longissimus dorsi* muscle.

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