Journal of Elementology ISSN 1644-2296

Fiedorowicz E., Sobotka W., Stanek M., Drażbo A. 2016. *The effect of dietary protein restriction in finishing pigs on the fat content, fatty acid profile, and atherogenic and thrombogenic indices of pork*. J. Elem., 21(3): 693-702. DOI: 10.5601/jelem.2015.20.2.949

THE EFFECT OF DIETARY PROTEIN RESTRICTION IN FINISHING PIGS ON THE FAT CONTENT, FATTY ACID PROFILE, AND ATHEROGENIC AND THROMBOGENIC INDICES OF PORK*

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Abstract

The objective of this study was to determine the effect of dietary protein restriction in finishing pigs on the fat content, fatty acid profile, and atherogenic and thrombogenic indices of *m. longissimus dorsi*. A feeding trial was performed on 45 crossbred [(Polish Landrace x Polish Large White) x Duroc] finishing pigs with an average initial body weight of 65 kg. The animals were divided into experimental groups and were fed finisher diets with different levels of total protein and total lysine: group S-c- standard protein level, O – protein level reduced by 15% relative to the standard level, O+AA – protein level reduced by 15% and supplemented with crystalline lysine to the standard level. The intramuscular fat content, fatty acid profile and health-promoting properties of *m. longissimus dorsi* were determined. A 15% reduction in total protein levels in diets for finishing pigs insignificantly increased the intramuscular fat content of *m. longissimus dorsi,* but had no influence on the total concentrations of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The supplementation of low-protein diets with crystalline lysine decreased the intramuscular fat content of *m. longissimus dorsi*, decreased the concentrations of margaric-oleic acid (C17:1) and gadoleic acid (C 20:1), and increased α–linolenic acid (C18:3) levels. Meat from pigs fed a low -protein diet supplemented with lysine was characterized by a lower (more desirable) *n*-6/*n*-3 PUFA ratio and provide more atherogenic and trombogenic properties.

Keywords: pigs, low protein diet, intramuscular fat, fatty acids, atherogenic index, thrombogenic index.

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* This work was financially supported by the Ministry of Science and Higher Education, Project No. N N311 25 53 36.

INTRODUCTION

Meat is an important component of the human diet. It is a rich source of readily available protein with a high biological value and desirable essential amino acid composition (Higgs 2000). Meat contains minerals such as iron, zinc, phosphorus, magnesium, copper and sulfur, and B-group vitamins that are involved in nervous system function and play important roles in carbohydrate, fat and protein metabolism (Biesalski 2005). Meat also contains fat, SFAs and cholesterol, which are claimed to exert adverse health effects. According to Migdał et al. (2008), the consumption of large amounts of meat and animal fats can shorten life expectancy. A high-fat diet has also been linked to obesity and colorectal cancer. Foods high in saturated fats and cholesterol are risk factors for cardiovascular disease (Jiménez-Colmenero et al. 2001). However, fat is required for phospholipid synthesis. Linoleic acid, found in the lipids of cell membranes, contributes to reducing adipose tissue and blood cholesterol levels (Webb, O'Neill 2008). According to the FAO recommendations (2009), the intake of total dietary fat in adults should range from 20 and 35% energy, which means that 20-35% of the total daily energy intake should come from dietary sources of fats. Total SFA consumption should not exceed 10% energy.

In recent years, pig producers have attempted to reduce carcass fat content and change the fatty acid profile of pork in order to achieve a higher carcass leanness (Czarniecka-Skubina et al. 2007). Intramuscular fat content is an important quality trait that affects the flavour, juiciness and tenderness of meat. Since intramuscular fat content below 1% may have a negative influence on the flavour and eating quality of pork, the recommended content is 2% to 2.5-3% (Jankowiak et al. 2010). Recent years have witnessed an increasing interest in modifications of the fatty acid composition of both muscle and adipose tissue to produce meat with desirable nutritional and technological properties. In monogastric animals such as pigs, dietary factors have a significant effect on the fatty acid profile of meat, which facilitates production of pork providing health benefits to consumers (Alonso et al. 2010, Flis et al. 2010, Sobotka et al. 2012). However, it should be noted that increasing concentrations of desirable PUFAs in meat increase lipid susceptibility to oxidation, which has adverse effects on the sensory properties of meat products and human health (Jiménez-Colmenero et al. 2001).

The aim of this study was to determine the effect of dietary protein restriction in finishing pigs on the fat content, fatty acid profile, and atherogenic and thrombogenic indices of *m. longissimus dorsi*.

MATERIAL AND METHODS

A feeding trial was performed on 45 crossbred [(Polish Landrace x Polish Large White) x Duroc] finishing pigs with an average initial body weight of 65 kg. The animals were divided into experimental groups by the analogue method, and they were fed finisher diets with different levels of total protein and total lysine, according to the experimental design presented in Table 1. The pigs were kept in single, individual pens with a slatted floor. Feed was rationed, and water was available *ad libitum*.

Table 1

Experimental design

Experimental factors	Experimental groups		
	S-c*	O	O + AA
Total protein level/total lysine level in experimental diets	standard protein level**	protein level reduced by 15% relative to the standard level	protein level reduced by 15% and supplemented with crystalline lysine to the standard level
(%)	15.0 / 0.78	12.72 / 0.67	12.79 / 0.78
Number of meat samples *(m. longisimus dorsi)*	15	15	15

* S-c – control group, ** balanced to achieve daily weight gain of 850 g (Frankiewicz 2014)

Finisher diets contained ground barley, ground wheat, soybean meal, 00-rapeseed meal, minerals and vitamins. The O+AA diet was supplemented with small amounts of crystalline methionine, threonine and tryptophan to achieve identical levels of those amino acids as in the control diet (S-c)

At the end of the feeding trial, pigs with the average body weight of 110 kg were slaughtered at the WARMIA Meat Processing Plant in Biskupiec near Olsztyn (NE Poland). After 24 hours of carcass chilling at 2-4°C, samples of *m. longissimus dorsi* were collected to determine intramuscular fat content by Soxhlet extraction (AOAC 2010). Fatty acid methyl esters were extracted by the Peisker method modified by Żegarska et al. (1991), and were separated by gas chromatography using the VARIAN CP-3800 system with a flame ionization detector (FID).

The atherogenicity and thrombogenicity of intramuscular fat from *m. longissimus dorsi* were determined based on the atherogenic index (AI) and thrombogenic index (IT), calculated from the formulas proposed by Ulbricht and Southgate (1991):

$$\text{IA} = \frac{C12{:}0 + 4C14{:}0 + C16{:}0}{PUFA\ n{-}3 + PUFA\ n{-}6 + MUFA}$$

$$\text{IT} = \frac{C14{:}0 + 4C16{:}0 + C18{:}0}{0.5MUFA + 0.5PUFA\ n{-}6 + 3PUFA\ n{-}3 + \frac{PUFA\ n{-}3}{PUFA\ n{-}6}}$$

The results were analyzed statistically by one-way ANOVA with the use of Statistica ver. 10 PL software. The study takes into account the results of statistical arithmetic mean ($\bar{x}$) and standard deviation (SD). The significance of differences between mean values of the analyzed experimental factors was estimated by the Duncan's test at the significance level $P \leq 0.05$ and $P \leq 0.01$.

RESULTS AND DISCUSSION

Table 2 presents the intramuscular fat content of *m. longissimus dorsi*, which was not significantly affected by dietary protein restriction. The 15% reduction in the total protein content of the experimental finisher diet (group O) relative to the control diet (group S-c) increased the intramuscular fat content of pork from 1.87% (group S-c) to 1.99% (group O). The supplementation of the low-protein diet with crystalline lysine, methionine, threonine and tryptophan (group O+AA) decreased the intramuscular fat content of pork to 1.80%. Our results corroborate the findings of Millet et al. (2006) and Senčić et al. (2011), who did not observe significant differences in the intramuscular fat content of *m. longissimus dorsi* in response to different crude protein levels in diets fed to growing-finishing pigs. In contrast, other authors (Teye et al. 2006, Wood et al. 2006, Alonso et al. 2010) noted highly significant differences in the intramuscular fat content of *m. longissimus dorsi* in pigs fed diets with graded inclusion levels of total protein. In our study, marbling was higher in pork from pigs fed low-protein diets, which - according to Alonso et al. (2010) - could result from limited protein synthesis and the increased amount of energy available for fat deposition.

Our analysis of the SFA profile of *m. longissimus dorsi* (Table 2) did not reveal significant differences between the groups. In a study by Alonso et al. (2010), there were no significant differences in the concentrations of palmitic acid (C16:0), stearic acid (C18:0) or total SFAs between the groups fed diets with an increased and decreased total protein content. Teye et al. (2006) demonstrated that the concentrations of myristic acid (C14:0), palmitic acid (C16:0) and total SFAs increased significantly along with an increase in the intramuscular fat content of pork. However, in studies by Więcek (2009), feeding restriction by 25% in fattening pigs during the first phase of fattening caused a significant reduction in C14: 0 compared to the control group.

Table 2

The concentrations of intramuscular fat and saturated fatty acids in *m. longissimus dorsi*

Specification	Statistical measures	Experimental groups*			Significance level P
		S-c	O	O+AA	
Intramuscular fat (%)	$\bar{x}$	1.87	1.99	1.80	0.66
	SD	0.50	0.61	0.39	
Saturated fatty acids (SFAs, % of the total FA pool)					
C 10:0	$\bar{x}$	0.15	0.15	0.15	0.33
	SD	0.01	0.01	0.01	
C 12:0	$\bar{x}$	0.11	0.11	0.11	0.77
	SD	0.01	0.01	0.01	
C 14:0	$\bar{x}$	1.67	1.68	1.65	0.87
	SD	0.08	0.13	0.10	
C 15:0	$\bar{x}$	0.05	0.06	0.05	0.12
	SD	0.01	0.02	0.01	
C 16:0	$\bar{x}$	29.84	29.74	29.55	0.71
	SD	0.97	0.97	0.70	
C 17:0	$\bar{x}$	0.26	0.25	0.21	0.17
	SD	0.06	0.08	0.03	
C 18:0	$\bar{x}$	14.96	14.59	15.04	0.40
	SD	0.94	0.97	0.95	
C 20:0	$\bar{x}$	0.20	0.18	0.20	0.16
	SD	0.03	0.02	0.02	
C 22:0	$\bar{x}$	0.05	0.06	0.06	0.73
	SD	0.02	0.02	0.02	
Total	$\bar{x}$	47.28	46.79	47.01	0.72
	SD	1.60	1.80	1.45	

* see Table 1, SD – standard deviation

The concentrations of MUFAs and PUFAs in *m. longissimus dorsi* are shown in Table 3. The low-protein diet (total protein levels reduced by 15% relative to the control diet) supplemented with limiting amino acids (lysine, methionine, threonine and tryptophan, group O+AA) led to a significant ($P \leq 0.05$) decrease (to 0.26%) in margaric-oleic acid (C17:1) concentrations. Gadoleic acid (C20:1) levels also decreased significantly ($P \leq 0.05$), from 0.82% in group S-c to 0.72% in group O+AA. No changes were observed in the concentrations of myristoleic acid (C14:1), palmitoleic acid (C16:1), oleic acid (C18:1) or total MUFAs. Different results were reported by Teye et al. (2006), who found that dietary protein levels had a significant effect on

Table 3

The concentrations of intramuscular fat and unsaturated fatty acids in *m. longissimus dorsi*

Specification		Statistical measures S-c	Experimental groups*			Significance level *P*
			O	O+AA		
Intramuscular fat		$\bar{x}$ s	1.87 0.50	1.99 0.61	1.80 0.39	0.66
Unsaturated fatty acids (% of the total FA pool)	fatty acids					
Monounsaturated fatty acids (MUFAs)	C 14:1	$\bar{x}$ SD	0.03 0.01	0.04 0.01	0.03 0.01	0.22
	C 16:1	$\bar{x}$ SD	4.35 0.50	4.62 0.44	4.47 0.45	0.28
	C 17:1	$\bar{x}$ SD	0.32^{a} 0.06	0.31^{a} 0.07	0.26^{b} 0.05	0.03
	C 18:1	$\bar{x}$ SD	42.96 1.23	43.11 1.48	43.00 1.08	0.95
	C 20:1	$\bar{x}$ SD	0.82^{a} 0.08	0.75 0.09	0.72^{b} 0.10	0.02
	total	$\bar{x}$ SD	48.47 1.56	48.83 1.70	48.48 1.34	0.79
Polyunsaturated fatty acids (PUFAs)	C 18:2	$\bar{x}$ SD	3.59 0.84	3.58 0.91	3.69 0.58	0.93
	C 18:3	$\bar{x}$ SD	0.23^{B} 0.04	0.27^{A} 0.06	0.29^{A} 0.04	<0.01
	C 20:2	$\bar{x}$ SD	0.16 0.03	0.15 0.03	0.16 0.03	0.72
	C 20:4	$\bar{x}$ SD	0.32 0.16	0.35 0.14	0.44 0.14	0.15
	total	$\bar{x}$ SD	4.30 1.02	4.36 1.08	4.58 0.75	0.76
UFAs** SD		$\bar{x}$ 1.65	52.78 1.87	53.19 1.37	53.07	0.79

a, *b* – mean values in the same line with different superscript letters differ significantly at $P \leq 0.05$, *A*, *B* – mean values in the same line with different superscript letters differ significantly at $P \leq 0.01$, SD – standard deviation, * see Table 1, ** UFAs = MUFAs + PUFAs

individual and total MUFAs in pork. Alonso et al. (2010) also noted a significant increase in the concentrations of C20:1 fatty acid and total MUFAs in the meat of pigs fed a commercial diet with a reduced total protein content.

Diets in which the total protein content was reduced by 15% (group O) relative to control group S-c, and which were enriched with lysine, methionine, threonine and tryptophan (group O+AA) contributed to a significant ($P \leq 0.01$) increase in αlinolenic acid (C18:3) concentrations in the intramuscular fat of *m. longissimus dorsi,* from 0.23% in group S-c to 0.27% in group O and 0.29% in group O+AA. Our results are consistent with the findings of Teye et al. (2006) and Alonso et al. (2010), who also noted a significant effect of dietary protein levels on αlinolenic acid (C18:3) concentrations in pork. In the cited studies, the experimental factor had a significant effect on total PUFAs, which was not observed in our experiment. Both MUFAs and PUFAs lower the serum levels of low-density lipoproteins – LDL (Mattson, Grundy 1985).

In our study, the n-6/n-3 PUFA ratio was more desirable in pork from group O pigs fed a diet with total protein levels reduced by 15% relative to the control group (Table 4). The *n*-6/*n*-3 PUFA ratio was further improved in group O+AA where a low-protein diet was supplemented with essential ami-

Table 4

Health-promoting properties of intramuscular fat from *m. longissimus dorsi*

Specification	Statistical measures	Experimental groups*			Significance level P
		S-c	O	O+AA	
n-3	$\bar{x}$	0.23^B	0.27^A	0.29^A	<0.01
	SD	0.04	0.06	0.04	
n-6	$\bar{x}$	3.91	3.93	4.13	0.82
	SD	0.97	1.01	0.70	
n-6/*n*-3	$\bar{x}$	17.00^A	14.55^B	14.24^B	<0.01
	SD	3.11	2.52	1.07	
DFAs**	$\bar{x}$	67.73	67.76	68.11	0.57
	SD	1.00	1.06	0.75	
OFAs***	$\bar{x}$	32.32	32.22	31.97	0.66
	SD	1.02	1.03	1.80	
DFA/OFA	$\bar{x}$	2.10	2.11	2.13	0.64
	SD	0.09	0.10	0.08	
PUFA/SFA	$\bar{x}$	0.09	0.09	0.10	0.77
	SD	0.02	0.03	0.02	
AI****	$\bar{x}$	0.70	0.69	0.68	0.82
	SD	0.04	0.05	0.04	
TI*****	$\bar{x}$	1.20	1.18	1.16	0.75
	SD	0.13	0.15	0.10	

A, *B* – mean values in the same line with different superscript letters differ significantly at $P \leq 0.01$, SD – standard deviation, * see Table 1, ** DFAs = UFAs + C18:0, *** OFAs = SFAs – C18:0, **** AI – atherogenic index, ***** TI – thrombogenic index

no acids. The differences between experimental groups (O and O+AA) and the control group (S-c) were statistically significant ($P \leq 0.01$). Despite the beneficial influence of the experimental factor, the *n*-6/*n*-3 PUFA ratio was high, ranging from 14.24 to 14.47. The noted values are typical of animal fats and considerably different from the recommended *n*-6/*n*-3 PUFA ratio of 4-5:1 in the human diet.

The dietary levels of total protein and essential amino acids had no effect on the concentrations and proportions of hypocholesterolemic fatty acids (DFAs) and hypercholesterolemic fatty acids (OFAs). The PUFA/SFA ratio was unaffected by the experimental factor, either, which corroborates previous findings (Teye et al. 2006, Alonso et al. 2010). However, the studies carried out by Więcek (2009) showed a significantly higher ratio of these acids using a feed restriction reduced by 25% relative to the control group. The PUFA/SFA ratio recommended by the UK Department of Health (*Nutritional* … 1994) is 0.4-0.5. The values noted in our study were lower (0.09-0.10) in both experimental groups, due to low PUFA concentrations in the intramuscular fat of *m. longissimus dorsi*.

A statistically non-significant decrease was observed in the atherogenicity (AI) and thrombogernicity (TI) of intramuscular fat from *m. longissimus dorsi* samples collected from finishing pigs fed a low-protein diet (group O). Dietary supplementation with crystalline lysine (group O+AA) contributed to further decrease in the above indices. Our findings are consistent with those of Hu (2001) and Henderson et al. (2008), who found that fats with increased concentrations of unsaturated fatty acids exerted antiatherogenic and antithrombogenic effects.

CONCLUSIONS

1. A 15% reduction in total protein levels in diets for finishing pigs insignificantly increased the intramuscular fat content of *m. longissimus dorsi,* but had no influence on the total concentrations of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs).

2. The supplementation of low-protein diets with crystalline lysine decreased the intramuscular fat content of *m. longissimus dorsi*, decreased the concentrations of margaric-oleic acid (C17:1) and gadoleic acid (C 20:1), and increased α-linolenic acid (C18:3) levels.

3. Meat from pigs fed a low-protein diet supplemented with lysine was characterized by a lower (more desirable) *n*-6/*n*-3 PUFA ratio and provide more atherogenic and trombogenic properties.

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