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FATTY ACIDS PROFILE OF VARIOUS MUSCLES OF PIGS FED IN THE FIRST PERIOD OF FATTENING WITH RESTRICTIVE OR *SEMI AD LIBITUM* DIETS

Justyna Więcek

Department of Pig Breeding, Warsaw University of Life Sciences

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A study conducted on 24 fatteners was undertaken to determine the effect of the level of feeding pigs in the first period of fattening on fatty acids profile of their muscles. In animals with body weight (b.w.) of 23 kg, the content of crude fat was similar in all muscles examined. In a lipid fraction of *M. longissimus thoracis, M. semimembranosus* and heart muscle of pigs with b.w. of 23 kg and 60 kg, analyses demonstrated statistically significant differences in fatty acids profile. In the heart muscle, as compared to the skeletal muscles, the contribution of acids from SFA and MUFA families was lower ($p \leq 0.01$), and that of n-6 and n-3 PUFA acids was higher ($p \leq 0.01$). In each of the muscles examined, the contribution of saturated and monounsaturated fatty acids was lower and that of n-6 and n-3 polyunsaturated acids was higher in the animals fed a restrictive diet (75% of the dose fed to the control group) as compared to the fatteners fed *semi ad libitum*. It affected an increase in the ratio of polyunsaturated to saturated fatty acids in the first group of animals ($p \leq 0.01$). In fatteners fed a restrictive diet *vs.* those fed *semi ad libitum*, the content of total fat was lower in *longissimus thoracis* and *semimembranosus* muscles. That dependency was not observed in the heart muscle.

INTRODUCTION

Species is the main indicator of variability of fatty acids composition [de Smet *et al.*, 2004]. Meat of the ruminants is characterised by a higher concentration of fatty acids than that of the monogastric animals [Wood *et al*., 2008]. It results from the process of bihydrogenation of unsaturated fatty acids by microorganisms colonizing the rumen [Radzik-Rant, 2005]. The fatty acids profile is also affected by: breed, sex, maintenance conditions, feeding level and diet composition [Andrés *et al*., 2001; Wood *et al*., 2004; Nuernberg *et al.,* 2005; Mieńkowska-Stępniewska *et al*., 2006; Więcek *et al*., 2008a,b].

Tissues of an organism differ in terms of fatty acids composition [Doichev *et al*., 2003; Nuernberg *et al.,* 2005]. In the muscle tissue, differences occur even between muscles [Andrés *et al*., 2001; Doichev *et al*., 2003]. They are due to a diverse structure of muscles. In the skeletal muscles, red and white muscle fibres are arranged next to one another in a specified ratio, depending on the functional character of the muscles. The red fibres are characterised by slower contractility, but are more resistant than the white ones. They occur in muscles that perform more work [Przespolewska *et al*., 2009]. In the structure of *M. longissimus dorsi* analyses by Doichev *et al*. [2003], the contribution of the dark fibres accounted for 36%, whereas in *M. semimembranosus* – for 47%. It was additionally linked with changes in the content of muscle fat and fatty acids profile of triacylglycerol. Irrespective of a diet fed to pigs, more total fat and fatty acids of the PUFA family were demonstrated in *M. semimembranosus* than in *M. longissimus dorsi*. As reported by Gondret & Lebret [2007], muscles performing more work have the capability to accumulate lipids as a substrate for the production of energy.

A specific organism in a body that performs continuous work is the heart muscle. Its conduction system is built of fibres containing little contracting elements and much sarcoplasma (red fibres) [Przespolewska *et al*., 2009]. The fatty acid profile of that muscle, as compared to other muscles and fat, is characterised by a high contribution of fatty acids of the PUFA family [Nuernberg *et al*., 2005].

The objective of the presented study was to determine the effect of feeding level of pigs on fatty acids profile of their muscles.

MATERIALS AND METHODS

The experiment was conducted on 24 fatteners (12 barrows and 12 gilts) crossbreds (F_1 sow (Polish Large White x Polish Landrace) x duroc boar). It was began at the body weight of the animals reaching *ca.* 23 kg. At the beginning of the experiment, 8 animals (gilts:barrows, 1:1) were slaughtered in order to determine the parameters examined before the introduction of an experimental factor. The other 16 fatteners were allocated, based on analogs, to two groups: A and R. In group A, the pigs were fed *semi ad libitum*, whereas in group R – a restrictive diet, *i.e*. at a level of 75% of a diet fed to group A. The animals were fed individually with a feed mixture composed of wheat and barley meal, wheat bran, extracted soybean meal, mineral-vitamin mixes and crystalline

Author's address for correspondence: Justyna Więcek, Department of Pig Breeding, Warsaw University of Life Sciences, ul. Ciszewskiego 8, 02-786 Warszawa, Poland, tel.: (48 22) 59 365 63; fax: (48 22) 853 09 14; e-mail: justyna_wiecek@sggw.pl

amino acids. The nutritive value of 1 kg of the feed mixture reached 12.5 MJ ME; it contained 153 g of total protein and 9.6 g of lysine. The animals were slaughtered after the termination of the first fattening period, at live body weight of 60 kg. Results presented in this manuscript are a part of a greater research project.

From all slaughtered fatteners (24 animals), sections of: heart muscle, *M. longissimus thoracis* from the area of the penultimate thoracic vertebra in the cephalic direction, and *M. semimembranosus*, were collected after 24-h chilling. The material was comminuted and determined for the content of crude fat [AOAC, 1994]. The preparation and analysis of fatty acid methyl esters in a lipid fraction of muscles were conducted by means of the gas chromatography method [ISO 5509:1996; ISO 5508:1996] using a gas chromatograph Hewlett Packard 6890 Series GC System with a FID detector, a BPX 70 capillary column (50 m x 0.25 mm i.d; phase thickness – 0.25 μm; carrier gas – helium). Particular peaks were expressed in per cents of the total content of fatty acids. Results obtained were elaborated statistically using a one-way (effect of muscle) or two-way (effect of muscle, feeding level and interactions of those factors) analysis of variance with the least squares method [SPSS, 2006].

RESULTS

In the animals slaughtered at the beginning of the experiment, the content of crude fat was at a similar level in all muscles (Table 1). The lipid fraction of the heart muscle, as compared to that of *M. longissimus thoracis* and *M. semimembranosus*, was characterised by a higher content of saturated and monounsaturated fatty acids, both of the n-6 and n-3 family. The greatest differences were reported for: C 14:0 and C 16:1 acids (*ca*. -50%), as well as for C 18:1 (*ca*. -70%), C 18:2 (*ca*. +50%), C 20:4 (over three time more), C 20:5 (over two times more), and C 22:5 (*ca*. +50%). In turn, smaller differences were observed in fatty acids profile between *M. longissimus thoracis* and *M. semimembranosus.* In *M. semimembranosus*, as compared to *M. longissimus thoracis*, analyses demonstrated lower concentrations of: palmitic ($p\leq0.05$), docosatetraenoic ($p\leq0.01$), and eicosapentaenoic acid ($p\leq0.05$), as well as a lower concentration of linolenic acid ($p\leq0.01$). In all muscles, the ratio of n-6 to n-3 PUFA acids was high and reached over 13. In *M. longissimus thoracis* and *M. semimembranosus*, the PUFA/SFA ratio accounted for *ca*. 0.5, whereas in the cardiac muscle its was twice as high.

In the skeletal muscles of the animals fed a restrictive diet in the Ist period of fattening, as compared to those fed *semi ad libitum*, the content of crude fat was found to be lower (Table 2). Such a dependency was not observed in the heart muscle. All muscles of animals from group R, as compared to those of the fatteners from group A, were characterised by a lower contribution of SFA and MUFA and a higher contribution of PUFA. The greatest differences were reported in *M. longissimus thoracis*, in which the level of n-6 and n-3 PUFA increased by 75% and 67%, in respect of group A. As compared to the skeletal muscles, in the heart muscle the contribution of SFA (especially of C 18:0 acid), n-6 and n-3 PUFA was higher and that of MUFA was lower ($p\leq0.01$). Irrespective of the level of feeding, the ratio of n-6 to n-3 polyunsaturated fatty acids accounted for *ca*. 10 in all muscles. In the animals fed the restrictive diet, irrespective of the muscle, the PUFA:SFA ratio was higher ($p\leq0.01$) than in the fatteners fed *semi ad libitum*.

TABLE 1. Fatty acids profile of different muscles (%) (b.w. 23 kg).

Specification	Muscle			SE
	Longissimus thoracis	*Semimembranosus*	Heart	
Crude fat	1.10	1.15	1.09	0.09
SFA	39.45^{Aa}	37.36^{b}	36.44^{B}	0.37
C 14:0	1.11^{A}	1.27^{A}	0.58^{B}	0.04
C 16:0	25.59^{Aa}	24.30^{Ab}	20.89^{B}	0.23
C 18:0	12.75^{a}	11.79^{A}	14.98^{Bb}	0.38
MUFA	42.08^{A}	43.96^{A}	29.92^{B}	0.66
C 16:1	3.74^{A}	4.14^{A}	2.05^{B}	0.16
C 18:1	38.34^{A}	39.82^{A}	27.88^{B}	0.63
PUFA n-6	17.17^{A}	17.42^{A}	32.01^{B}	0.72
C 18:2	13.41^{A}	14.64^{A}	21.07^{B}	0.41
C 20:4	3.05^{A}	2.31^{A}	10.31^{B}	0.35
C 22:4	0.71^{A}	0.48^{aB}	0.63^{b}	0.03
PUFA n-3	1.30^{a}	1.26^{a}	1.62^{b}	0.05
C 18:3	0.61^{Aa}	0.75^{B}	0.48^{Ab}	0.02
C 20:5	0.20^{Aa}	0.11^{Ab}	0.44^{B}	0.02
C 22:5	0.49^{A}	0.40^{A}	0.70^{B}	0.03
PUFA n-6/n-3	13.24^{A}	13.92^{A}	19.91^{B}	0.28
PUFA/SFA	0.47^{A}	0.50^{A}	0.93^{B}	0.03

$^{a, b}$ – $p\leq0.05$; $^{A, B}$ – $p\leq0.01$

TABLE 2. Contents of fat and profile of fatty acids (%) (b.w. 60 kg).

Specification	Muscle	Level of feeding		Mean	SE
		A	R		
Crude fat	*L. thoracis*	1.11	0.64	0.88	
	semimembranosus	1.36	1.05	1.20	
	heart	1.05	1.10	1.08	
	mean	1.17	0.93	1.05	0.06
SFA	*L. thoracis*	39.20	36.69	37.95^{A}	
	semimembranosus	38.68	37.04	37.86^{A}	
	heart	43.56	39.78	41.67^{B}	
	mean	40.48^{A}	37.84^{B}	39.16	0.24
C 14:0	*L. thoracis*	1.22	0.98	1.10	
	semimembranosus	1.25	1.09	1.17	
	heart	1.17	1.01	1.09	
	mean	1.21^{A}	1.02^{B}	1.12	0.02
C 16:0	*L. thoracis*	25.28	24.05	24.67^{a}	
	semimembranosus	24.71	23.73	24.22	
	heart	24.95	22.71	23.83^{b}	
	mean	24.98^{A}	23.50^{B}	24.24	0.13
C 18:0	*L. thoracis*	12.70	11.66	12.18^{A}	
	semimembranosus	12.73	12.22	12.47^{A}	
	heart	17.44	16.07	16.75^{B}	
	mean	14.29^{A}	13.32^{B}	13.80	0.15
MUFA	*L. thoracis*	49.93	44.31	47.12^{A}	
	semimembranosus	49.19	45.38	47.28^{A}	
	heart	39.21	35.32	37.27^{B}	
	mean	46.11^{A}	41.67^{B}	43.89	0.36
C 16:1	*L. thoracis*	3.95	3.50	3.73^{A}	
	semimembranosus	3.76	3.50	3.63^{A}	
	heart	2.43	2.16	2.30^{B}	
	mean	3.38^{a}	3.06^{b}	3.22	0.06
C 18:1	*L. thoracis*	45.98	40.81	43.40^{A}	
	semimembranosus	45.43	41.88	43.65^{A}	
	heart	36.78	33.16	34.97^{B}	
	mean	42.73^{A}	38.62^{B}	40.67	0.34
PUFA n-6	*L. thoracis*	9.87	17.32	13.60^{A}	
	semimembranosus	11.07	15.90	13.49^{A}	
	heart	15.71	22.97	19.34^{B}	
	mean	12.22^{A}	18.73^{B}	15.47	0.44
C 18:2	*L. thoracis*	7.74	12.94	10.34^{A}	
	semimembranosus	9.07	12.42	10.75^{A}	
	heart	12.04	16.47	14.26^{B}	
	mean	9.62^{A}	13.94^{B}	11.78	0.30
C 20:4	*L. thoracis*	1.70	3.60	2.65^{A}	
	semimembranosus	1.61	2.83	2.22^{A}	
	heart	3.26	5.95	4.61^{B}	
	mean	2.19^{A}	4.13^{B}	3.16	0.14
C 22:4	*L. thoracis*	0.44	0.78	0.61^{a}	
	semimembranosus	0.39	0.65	0.52	
	heart	0.40	0.54	0.47^{b}	
	mean	0.41^{A}	0.66^{B}	0.54	0.02
PUFA n-3	*L. thoracis*	1.00	1.67	1.34^{A}	
	semimembranosus	1.06	1.68	1.37^{A}	
	heart	1.53	1.93	1.72^{B}	
	mean	1.20^{A}	1.76^{B}	1.48	0.04
C 18:3	*L. thoracis*	0.50	0.69	0.60^{A}	
	semimembranosus	0.66	0.92	0.79^{B}	
	heart	0.89	0.81	0.85^{B}	
	mean	0.68^{A}	0.81^{B}	0.74	0.02*
C 20:5	*L. thoracis*	0.18	0.32	0.25a	
	semimembranosus	0.13	0.26	0.20^{Bb}	
	heart	0.20	0.36	0.28^{A}	
	mean	0.17^{A}	0.31^{B}	0.24	0.01
C 22:5	*L. thoracis*	0.32	0.66	0.49^{a}	
	semimembranosus	0.27	0.50	0.39^{Ab}	
	heart	0.43	0.76	0.60^{Bb}	
	mean	0.34^{A}	0.64^{B}	0.49	0.02
PUFA n-6/n-3	*L. thoracis*	9.90	10.35	10.12^{a}	
	semimembranosus	10.42	9.47	9.94^{A}	
	heart	10.28	12.02	11.15^{Bb}	
	mean	10.20	10.61	10.41	0.16*
PUFA/SFA	*L. thoracis*	0.28	0.52	0.40^{A}	
	semimembranosus	0.32	0.47	0.39^{A}	
	heart	0.40	0.63	0.52^{B}	
	mean	0.33^{A}	0.54^{B}	0.44	0.01

$^{a, b}$ – $p \leq 0.05$; $^{A, B}$ – $p \leq 0.01$; * – interaction muscle x level of feeding

DISCUSSION

The fatty acids profile determined in the lipid fraction of the heart muscle of animals with body weight of 23 kg and 60 kg differed significantly than that assayed in the *longissimus thoracis* and *semimembranosus* muscles. In the heart muscle, as compared to other tissues, analyses showed considerably lower concentrations of fatty acids from the MUFA family, and higher concentrations of those from the n-6 and n-3 PUFA family. It is consistent with results achieved by Nuernberg *et al*. [2005], who when examining the effect of olive oil and linseed oil on the fatty acid profile of *longissimus* muscle, backfat and heart muscle, demonstrated a significantly higher contribution of polyunsaturated fatty acids in the fatty acid profile of the heart muscle, irrespective of the oil applied. Their study indicated that enriching fatteners diets with the addition of olive oil as a source of monounsaturated fatty acids and of linseed oil as a source of polyunsaturated fatty

acids of the n-3 family affected concentrations of individual PUFA acids of n-6 and n-3 family in the heart muscle, yet changed the sum of PUFA only to a small extent. As compared to the other tissues, the heart muscle turned out to be more "stable", presumably owing to its function served in the body. In the reported experiment, the restricted level of pigs feeding affected a change in the fatty acid profile of the heart muscle, with the change being lesser as compared to the skeletal muscles.

Very stable, irrespective of the level of feeding, appeared to be the total fat content of the heart muscle. This is likely to be linked with the work of that muscle, in which the accumulated lipids are utilized continuously as a substrate for the production of energy [Gondret & Lebret, 2007]. Insufficient supply of feed mixtures resulted in a reduced content of total fat in the skeletal muscles, but the extent of changes was considerably greater in *M. longissimus thoracis* than in *M. semimembranosus.* Irrespective of the experimental factor, in the reported study as well as in experiments conducted by Doichev *et al.* [2003] and Migdał *et al.* [2007], a higher content of total fat was demonstrated in *M. semimembranosus vs. M. longissimus thoracis*. It is most probably due to a greater capability of fat deposition observed in the red fibres [Gondret & Lebret, 2007], which occur in a higher number in *M. semimembranosus* than in *M. longissimus thoracis* [Doichev *et al.,* 2003].

In the fatty acids profile of *longissimus thoracis* and *semimembranosus* muscles, the analyses did not demonstrate so great differences as in their comparison with the heart muscle. *M. semimembranosus* was characterised by a lower content of SFA and by a considerably higher content of n-6 PUFA as compared to *M. longissimus thoracis*. Irrespective of the body weight of slaughtered fatteners, also Stasiak *et al.* [2007] demonstrated a higher contribution of essential fatty acids (EFA) in the fatty acids profile of ham as compared to loin. In the reported experiment, despite a high effect of the level of feeding on the fatty acids profile of both these muscles, a smaller extent of changes was observed in the lipid fraction of *M. semimembranosus vs. M. longissimus thoracis.* It is, however, inconsistent with results achieved by Migdał *et al*. [2000], according to whom fat of *M. longissimus dorsi* is more stable than fat of *M. semimembranosus* and responds to a high content of EFA in a feed mixture to a lesser extent.

The study demonstrated a positive, from the dietetic point of view (less SFA, more PUFA), effect of the restricted level of feeding on the fatty acids profile of all muscles. Alike dependencies were observed by Daza *et al*. [2007] in the inner and outer layer backfat of fatteners fed at a level of 50% of the diet administered to the control group. The authors demonstrated that the insufficient supply of energy with a diet suppressed the activity of lipogenic enzymes, thus contributing to impaired synthesis of saturated and monounsaturated fatty acids. This, in turn, determines a higher contribution of the unsaturated fatty acids in the total fatty acid profile. A low fat deposition results in the percentage contribution of PUFA [Biedermann *et al*., 2000; Daza *et al*., 2006, 2007; Więcek *et al*., 2008a].

Works of Wood *et al*. [2004] and Daza *et al*. [2006] confirmed the effect of the level of feeding on the fatty acids profile of various tissues; yet that effect was not always the same as in the reported experiment. The differences resulted from the level of restrictive feeding as well as from the type of tissues and lipid fractions, the profile was assayed in. Muscle lipids include primarily triacylglycerols and phospholipids [de Smet *et al*. 2004]. The content of triacylglycerols is determined by the total content of fat and ranges from 0.2% to over 5% of muscle mass. Phospholipids content of the muscles is relatively constant and independent of breed, feeding, age nor adiposity. It constitutes from 0.2% to 1% of muscle mass. Phospholipids are constituents of cellular membranes and differ in the fatty acids profile from triacylglycerols, being the major energetic material of an organism. Phospholipids are especially rich in PUFA (over 40%), whereas in triacylglycerols the contribution of PUFA reaches up to 15%. While feeding fatteners a low-protein diet (16% *vs.* 20% of total protein), Wood *et al*. [2004] demonstrated a higher content of linolenic acid in phospholipids, yet a lower one in neutral lipids of *longissimus dorsi* and *psoas major*. In turn, when applying restrictive feeding at a level of 75% of the diet of the control group, Cameron *et al*. [2000] observed higher concentrations of n-6 and n-3 polyunsaturated fatty acids in neutral lipids of *M. longissimus lumborum*. In phospholipids, those dependencies were opposite. In fatteners fed for 36 days a feeding dose reduced by 25%, Daza *et al*. [2006] reported lower contents of saturated fatty acids in the outer layer backfat and higher ones in the winner backfat layer.

In addition, the reported study demonstrated a higher contribution of PUFA in the younger animals (b.w. 23 kg) than in the older fatteners (b.w. 60 kg). Feeding restrictions were observed to change the ratio of polyunsaturated to saturated fatty acids. In all muscles, their ratio was found to increase, which may be recognized as a positive effect of the feeding factor. Wood *et al*. [2008] reports that the PUFA:SFA should reach >0.4, whereas according to Raes *et al*. [2004] it should account for >0.7. From the dietetic point of view, the n-6:n-3 PUFA ratio is of significance to a consumer. As indicated by Raes *et al.* [2004], it should reach <5. In the reported experiment, irrespective of the level of feeding and muscle examined, it accounted for *ca*. 10. The restricted feeding caused an increase in the contribution of not only n-3 polyunsaturated fatty acids but also of those from the n-6 family, which had a positive effect on the n-6:n-3 fatty acids ratio. Although n-6 and n-3 PUFA are metabolized by identical enzymatic systems [Raes *et al*., 2004], none of the members of both these families may be transferred into an acid belonging to other family. As it results from *in vitro* studies on hepatocytes, PUFA of the n-3 and n-6 families compete for desaturating enzymes, but still Δ4 and Δ6 desaturases utilize more readily n-3 than n-6 PUFA. Hence, a high ratio of C18:2 (n-6) to C18:3 (n-3) acid is likely to increase deficits of long-chain n-3 PUFA (*e.g.* C 22:5 and C 22:6) [Bartnikowska & Obiedziński, 1997].

CONCLUSIONS

Results of the conducted experiment enable concluding that, irrespective of the body weight of the fatteners, their muscles differed in the fatty acids profile. The heart muscle, as compared to the skeletal muscles, was characterized by

a considerably lower content of MUFA, and a higher concentration of n-6 and n-3 PUFA. In the lipid fraction of all analysed muscles of the fatteners fed a restrictive diet, as compared to those fed *semi ad libitum*, the contribution of saturated and monounsaturated fatty acids was lower, whereas that of n-6 and n-3 polyunsaturated fatty acids was higher. The level of feeding was found to affect the ratio of polyunsaturated to saturated fatty acids, but it had not significant effect on the ratio of n-6 to n-3 polyunsaturated fatty acids. The restricted level of feeding decreased the total fat content of the skeletal muscles.

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