

FATTY ACID COMPOSITION OF FAT TISSUE TRIGLICERIDES AND SKELETAL MUSCLE TISSUE HISTOSTRUCTURE OF PIGS FED DIET CONTAINING FLAX SEED

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Abstract. Fatty acid composition of muscle triacylglycerols and micro morphological structure of *m. longissimus thoracis et lumborum* and *m. semimembranosus* were determined. Linolenic acid (C18:3) level in *m. longissimus* triacylglycerols significantly (0.5%, 1.2% and 2.1%) increased for I, II and III group respectively. Omega ratio expressed as ratio C18:2/C18:3 in fat at *m. longissimus* was 9.0, 4.7 and 2.3 respectively for the three groups. Linolenic acid level in fat at *m. semimembranosus* rose significantly (0.4%, 1.8% and 2.6%) and omega ratio was 15.75, 3.78 and 2.58 respectively for I, II and III group. No statistically significant differences were found in mean diameter of muscle fibers, in ratio between dark and light muscle fibers and content of different tissue components in studied muscles caused by including of flax seed in the diets.

Key words: fatty acid composition, flax seed, micro morphological structure, muscle, pigs

INTRODUCTION

Content and form of fatty tissue in swine skeletal muscle have a large importance for pork quality. Many of researchers found that a high fatty content of pork increases its sensory quality [Essen-Gustavsson et al. 1994, Jones et al. 1994, Rede et al. 1986, Suzuki et al. 1991]. At the same time fatty acid composition of intramuscular fat determines nutritional value of pork. Due to it a large number of investigations tracing the growing of n-3 PUFA level in the animal tissues, and thus in human foods have been performed during last years.

The main part of these investigations are focused on the influence of feeds rich in n-3 PUFA (flax seed, canola, fish oil) on fatty acid composition of tissue lipids, pork quality and fattening ability of pigs [Cunnane et al. 1990, Doichev et al. 1998, Irie and Sokyomoto 1992, Machev et al. 1997, Morgan et al. 1992, Warnants et al. 1994]. The investigations on influence of these feeds on skeletal muscle microstructure are scanty.

The aim of this study was to determine the influence of different levels of flax seed in swine diets on fatty acid composition of fat tissue tryglicerides and microstructure of m. longissimus thoracis and m. semimembranosus.

MATERIAL AND METHODS

Thirty Danubian White sows were used in the experiment. Animals on 35 kg live weight were divided into three groups averaged by age and live weight. Pigs up to 65 kg were fed a diet containing 13.5% CP and 12.369 MJ ME/kg. During the last two months of fattening (65-110 kg) the 4% and 8% shares of ground flax seed were added to the diets respectively to II and III group. The CP level was kept constant while the ME level was as follows II group 12.512 and III group 12.748 MJ/kg. The feeds were prepared in two-weeks intervals without antioxidant adding. Fatty acid composition of feeds is presented in Table 1.

Table 1. Fatty acid composition of feeds, %
Tabela 1. Skład kwasów tłuszczowych w paszy, %

Fatty acids Kwasy tłuszczowe	Flax seed level, % Udział siemienia lnianego, %		
	0	4	8
14:0	1.1	0.9	0.7
16:0	32.8	29.8	25.5
16:1	1.5	1.2	1.3
17:0	0.4	0.7	1.3
18:0	12.1	12.4	10.8
18:1	33.9	36.4	36.1
18:2	18.2	16.7	19.0
18:3	–	1.9	5.3
SFA*	46.4	43.8	38.3
UFA*	53.6	56.2	61.7
MUFA*	35.4	37.6	37.4
PUFA*	18.2	18.6	24.3

*Abbreviations used: SFA – saturated fatty acids, UFA – unsaturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

*Używane skróty: SFA – nasycone kwasy tłuszczowe, UFA – nienasycone kwasy tłuszczowe, MUFA – mononasycone kwasy tłuszczowe, PUFA – wielonasycone kwasy tłuszczowe.

The pigs were slaughtered at 110 kg live weight. The samples were taken from m. longissimus thoracis et lumbarum from the area above the last three thoraces vertebrae, as well as from the middle part of m. semimembranosus.

Total lipids were extracted from muscle tissue according to Bligh and Dyer [1959]. Triglycerides from the lipids were refined by a thin layer chromatography on silica gel G plates and a movable solvent system hexane:ether (80:20) according to Dimov and

Dimitrov [1978]. Fatty acid methyl esters were treated with 2% H₂SO₄ in methanol, using the method of Angelov [1994].

Micromorphological studies were made on transverse and longitudinal sections of the two muscles coloured by Sudan black and astringent karmin, then average diameter of muscle fibers, ratio between dark and light muscle fibers, as well as ratio between different tissue compounds, were determined according to Petrov [1982].

RESULTS AND DISCUSSION

Diet of I gr. (control) contained most saturated fatty acids (SFA) and least unsaturated fatty acids (UFA). Polyunsaturated fatty acids are presented from linolenic acid (C18:2) only. Adding of ground flax seed to the diets of decreased SFA level and increased UFA level, as the latter caused was made mainly by increasing of the linolenic acid (C18:3) level.

Fatty acid composition of triaciloglycerols from *m. longissimus thoracis* is presented in Table 2. Including of flax seed to the diet caused statistically significant increase of C18:3 level in II group ($P \leq 0.01$) and in III group ($P \leq 0.001$) compared with I group. Differences between II and III group are significant too ($P \leq 0.01$). The level of C18:2 stayed almost unchanged, which made omega ratio expressed as ratio between C18:2/C18:3 to a significant decrease from 9 in I group to 4.16 in II group and 2.19 in III group. Oleic acid showed a decreasing trend when the level of flax seed in the diet rose. The levels of other fatty acids changed slightly, independent of the flax seed level in the diet. Warnants et al. [1999] observed similar changes in fatty acid composition of *m. longissimus* triaciloglycerols, rising of C18:3 level without significant changes of C18:2 level. They used full-fat soybean as PUFA source. Moreover, our results confirm those observed by Leskanich et al. [1993] and Warnants et al. [1996].

Fatty acid composition of triaciloglycerols from *m. semimembranosus* are presented also in Table 2. Adding of ground flax seed to the diet caused a statistically significant increase of C18:3 level in this muscle, too. Its level rose in II group ($P \leq 0.01$) and in III group ($P \leq 0.01$) compared with I (control) group. The difference between II and III group is insignificant. The level of C18:2 stayed almost unchanged. Omega ratio decreased from 15.75 in I group to 3.78 in II group and 2.58 in III group. These fatty acid changes are in an agreement with the results observed by Cunnane et al. [1990] after including of flax seed in the diet.

No statistically significant differences between two muscles in triaciloglycerols fatty acid composition were found.

From the data presented in Table 2 it could be concluded that fatty acid changes in two muscles may be explained by a similar model. Increasing of PUFA level was caused by increasing of C18:3 level and this happened independently of MUFA level.

The average diameter of muscle fibers of two muscles is presented in Figure 1. There are no large differences in the muscle fiber diameter of *m. longissimus* between the groups (up to 2 μm). Comparing average diameter of muscle fibers of *m. semimembranosus* we found that pigs of II group had the largest diameter (82.3 μm), followed by III group (76.4 μm) and I group (66.3 μm). These differences were not statistically significant.

Table 2. Fatty acid composition of triglycerides of muscle tissue, %
Tabela 2. Skład kwasów tłuszczowych w triacyloglicerydach mięśni, %

Fatty acids Kwasy tłuszczowe	Groups – Grupy					
	I		II		III	
	M. Longissi- mus dorsi	M. Semi- membra- nosus	M. Longissi- mus dorsi	M. Semi- membra- nosus	M. Longissi- mus dorsi	M. Semi- membra- nosus
	N = 10	N = 4	N = 10	N = 3	N = 9	N = 4
	X ± Sx	X ± Sx	X ± Sx	X ± Sx	X ± Sx	X ± Sx
14:0	2.0 ± 0.3	1.6 ± 0.1	1.4 ± 0.1	1.2 ± 0.3	1.9 ± 0.4	1.8 ± 0.3
16:0	27.0 ± 0.8	27.3 ± 1.2	28.1 ± 1.1	26.8 ± 2.3	26.9 ± 1.0	26.3 ± 3.2
16:1	3.8 ± 0.2	3.0 ± 0.2	3.5 ± 0.3	3.2 ± 0.4	3.9 ± 0.2	2.8 ± 1.1
18:0	11.0 ± 0.5	10.3 ± 1.4	10.6 ± 0.9	10.5 ± 1.6	11.5 ± 0.8	11.7 ± 2.3
18:1	51.2 ± 0.8	51.1 ± 4.2	50.2 ± 1.2	49.7 ± 6.1	49.1 ± 1.4	48.1 ± 6.2
18:2	4.5 ± 0.3	6.3 ± 1.1	5.0 ± 0.9	6.8 ± 1.3	4.6 ± 0.8	6.7 ± 2.1
18:3	0.5 ± 0.1 ^a	0.4 ± 0.1 ^a	1.2 ± 0.2 ^b	1.8 ± 0.3 ^b	2.1 ± 0.2 ^c	2.6 ± 0.4 ^b
SFA*	40	39.2	40.1	38.5	40.3	39.8
UFA*	60	60.8	59.9	61.5	59.7	60.2
MUFA*	55	54.1	53.7	52.9	53	50.9
PUFA*	5	6.7	6.2	8.6	6.7	9.3

Values marked with different superscript, by muscles, differ significantly $P \leq 0.001$.

*See Table 1.

Istotności różnic pomiędzy grupami oznaczonymi literami.

*Patrz tabela 1.

It could be concluded, that an addition of ground flax seed (up to 8%) in fattening swine diets had no influence on the average diameter of muscle fibers.

The ratio between light and dark muscle fibers in studied muscles is presented in Table 3. No significant differences were found between groups in this ratio in m. longissimus. There was a trend for higher content of dark muscle fibers in m. semimembranosus in II group (50.01%) compared with III group (46.86%) and I group (44.38%), but these differences were not significant. Almost a similar content of the two types muscle fibers in m. longissimus and slightly higher content of dark muscle fibers in m. semimembranosus in II and III group was found. In comparison with I group we can conclude that adding of flax seed in fattening pigs diet did not influence the ratio between two types muscle fibers in studied muscles. A similar conclusion was drawn also by Essen-Gustavsson et al. [1994] who found that composition of muscle triacyloglycerols did not influence the metabolic profile of muscles.

Ratio of different tissue components is presented in Table 4. The content of muscular and connective tissue in m. longissimus was almost similar in the three groups. The content of fatty tissue was the lowest in II group (2.1%) compared with I group (3.7%) and III group (3.1%). These differences were not statistically significant. The differences in the content of muscular and connective tissue in m. semimembranosus are larger, but had nothing in common with flax seed level in the diet. All groups had more intramuscular fatty tissue in m. semimembranosus than in m. longissimus. A trend was found for lower content of fatty tissue in m. semimembranosus in II group (6.7%), compared with I group (8%) and III group (9.4%). The same trend was found in m. longissimus too.

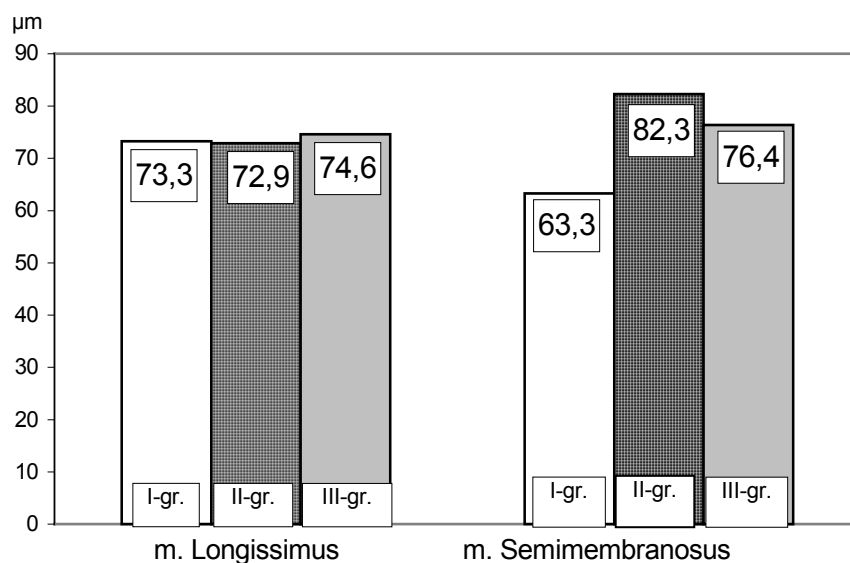


Fig. 1. Diameter of muscle fiber

Rys. 1. Grubość włókien mięśniowych

Table 3. Ratio between light and dark muscle fibers, %

Tabela 3. Procentowa zawartość ciemnych i jasnych włókien mięśniowych, %

Groups Grupy	M. Longissimus dorsi		M. Semimembranosus	
	light jasne	dark ciemne	light jasne	dark ciemne
I	64.67	35.33	55.62	44.38
II	62.07	37.93	49.99	50.01
III	66.47	33.53	53.14	46.86

Table 4. Ratio of muscle tissues, %

Tabela 4. Skład tkanki mięśniowej, %

Muscle Mięśnie	M. Longissimus dorsi			M. Semimembranosus		
	muscular tissue tkanka mięśniowa	connective tissue tkanka łączna	fatty tissue tkanka tłuszczowa	muscular tissue tkanka mięśniowa	connective tissue tkanka łączna	fatty tissue tkanka tłuszczowa
I group I grupa	79.2	17.1	3.7	75.1	16.9	8.0
II group II grupa	78.4	18.5	2.1	72.9	20.4	6.7
III group III grupa	77.8	19.1	3.1	78.2	12.4	9.4

Irrespective of differences in the content of intramuscular fatty tissue between the groups no significant differences were observed in disposition of this tissue in perimysium and endomysium of the muscles. It might be concluded, from the discussed results, that II group, with the lowest amount of intramuscular fatty tissue, had also darker muscle fibers.

CONCLUSIONS

1. Addition of 4% and 8% ground flax seed in the fattening pigs diets cause changes in fatty acid composition of triacyloglycerols from m. semimembranosus and m. longissimus. The level of linolenic acid rises significantly which causes significant decreasing of omega ratio expressed as ratio between C18:2/C18:3.

2. No significant differences were found in the average diameter of muscle fibers, in a content of different tissue components, as well as in the content of light and dark muscle fibers in studied muscles caused by flax seed level in the food.

REFERENCES

- Angelov A., 1994. Effect of vegetable oils in diets on contents and composition of tissue lipids in early weaned lambs. Dissertation Thracian University, Bulgaria, 47-48.
- Bligh E.G., Dyer W.J., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 8, 911-917.
- Cunnane S.C., Stit P.A., Gangull S., Armstrong J., 1990. Raised omega-3 fatty acid levels in pigs fed flax. *Can. J. Anim. Sci.* 70, 251-254.
- Dimov V., Dimitrov G., 1978. Investigation of nonesterified blood fatty acids. *Anim. Sci.* 15, 4, 92-98.
- Doichev V., Kirov M., Katzarov V., Ribarski S., Petrov P., 1998. Effect of ground flax seed in swine diets on pig performance, physico-chemical composition and quality of meat. *Bulg. J. Agric. Sci.* 4, 351-356.
- Essen-Gustavsson B., Karlsson A., Lundstrom K., Enfalt A., 1994. Intramuscular fat and muscle fiber lipid contents in halothan gene free pigs high or low protein diets and its relation to meat quality. *Meat Sci.* 38, 269-277.
- Jones S., Tong A.K., Campbell C., Dyck R., 1994. The effect of fat thickness and degree of marbling on pork colour and structure. *Can. J. Anim. Sci.* 74, 1, 155-157.
- Irie M., Sakamoto M., 1992. Fat characteristics of pigs fed fish oil containing eicosapentaenoic and docosahexaenoic acids. *J. Anim. Sci.* 70, 470-477.
- Leskanich C.O., Noble R.C., Morgan C.A., 1993. Manipulation of the polyunsaturated fatty acid content of pig meat in conformity with dietary guidelines. *Proc. Nutr. Soc.* 53, 14.
- Machev M., Vasileva M., Valchev G., Cvetkova V., Simovska M., 1997. Study of the effect of adding of polyunsaturated oils on pig growth, carcass composition and pork quality. *Anim. Sci.* 3-4, pp. 44-47.
- Morgan C.A., Noble R.C., Cocchi M., McCartney R., 1992. Manipulation of the fatty acid composition of pig meat lipids by dietary means. *J. Sci. Food Agric.* 58, 357-368.
- Petrov J., 1982. Species and breed particularities in microstructure of skeletal muscle during postnatal development of livestock animals. Dissertation Thracian University, Stara Zagora.
- Rede R., Pribisch V., Rahelic S., 1986. Untersuchungen uber die Beschaffenheit von Schlachtierkorpern und Fleisch primitiver und hochselektierter Schweinerassen. *Fleischwirtsch.* 66, 5, 898-907.

- Suzuki A., Kojima N., Ikeuchi Y., Ikarashi S., Moriyama N., Ishizuka T., Tokushige H., 1991. Carcass composition and meat quality of chinese purebred and European x Chinese crossbred pigs. *Meat Sci.* 29, 31-41.
- Warnants N., Oeckel M. J.V., Boucque Ch.V., 1994. The rate of incorporation of linoleic and linolenic acid in pork adipose tissue, as affected by feed and sex. *Medical Faculty Landbouww. University of Gent*, 59/4b.
- Warnants N., Van Oeckel M.J., Boucque C.V., 1996. Phospholipid and triglyceride fractions from pork muscle, as affected by dietary fat. In: *Proc. 42nd. ICOMST, Lillehammer, Norway*, 228-229.
- Warnants N., Van Oeckel M.J., Boucque C.V., 1999. Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. *J. Anim. Sci.* 77, 2478-2490.

SKŁAD KWASÓW TŁUSZCZOWYCH W TRIACYLOGLICERYDACH I MORFOLOGICZNA STRUKTURA MIĘŚNI SZKIELETOWYCH TUCZNIKÓW ŻYWIONYCH PASZĄ Z DODATKIEM SIEMIENIA LNIANEGO

Streszczenie. Oznaczono skład kwasów tłuszczowych w triacyloglicerydach i mikromorfologiczną strukturę mięśni: musculus longissimus lumborum i thoracis i m. semimembranosus. Poziom kwasu linolenowego (C18:3) w triacyloglicerydach mięśnia longissimus wzrósł istotnie w grupie II i III w stosunku do grupy kontrolnej. Dla trzech grup zwierząt współczynnik omega, wyrażony jako stosunek zawartości C 18:2/C 18:3 w mięśniu longissimus, wynosił odpowiednio 9,0, 4,7 i 2,3. Poziom kwasu linolenowego w tłuszczu mięśnia semimembranosus wzrósł znacząco (0,4, 1,8, 2,6%), a współczynnik omega wynosił 15,75, 3,78 i 2,58 odpowiednio dla grup I, II i III. Nie stwierdzono statystycznie istotnych różnic w przeciętnej grubości włókien mięśniowych i w porcjach pomiędzy włóknami jasnymi i ciemnymi.

Słowa kluczowe: kwasy tłuszczowe, siemię lniane, morfologia, tkanka mięśniowa, świnie

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