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LIPID PROFILE OF *M. LONGISSIMUS DORSI* IN RESPONSE TO DIETARY *SPIRULINA PLATENSIS* SUPPLEMENTATION IN PIGS

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Abstract

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The changes of the lipid profile and the fatty acid composition in the triacylglycerols and phospholipids in *m. Longissimus dorsi* (m. LD) of Danube White pigs in response to dietary Spirulina supplementation were studied. The experiment was divided in two sub periods, starting at 34 kg and ending at 102.5-106.3 kg live weight. The pigs in the trial were divided in three groups – control and two experimental. The pigs from the experimental groups were fed the same diet as the control group. The first experimental group (SP1) received additionally 2 g Spirulina platensis per capita daily only during the second sub period, while the pigs from the second experimental group (SP2) received the same amount (2 g) of Spirulina during the whole trial period. Dietary Spirulina supplementation did not influence the content of total lipids in m. LD although in the experimental groups they tended to increase. Significant decrease in the content of C14:0 in the triacylglycerols was observed in response to Spirulina supplement ($P < 0.05$) while in the phospholipid fraction the amount of C18:3n-6, C20:4 and C20:5 showed significant increase ($P < 0.05$). The content of C18:3n-3 and C22:5 tended to increase in m. LD of the pigs from the experimental groups.

Key words: *Spirulina platensis*, pigs, muscles, fatty acid composition

Introduction

Spirulina (*Arthrospira platensis*) is a filamentous cyanobacteria, that belongs to microalgae. Recently it has been drawing considerably the attention of the researchers due to its high nutritional value and functional properties. Spirulina is known to be a rich source of protein and bioactive compounds such as carotenoids. It has also high content of polyunsaturated fatty acids, the major part of which is formed by γ- linolenic acid (Cohen et al., 1987). This fatty acid has been known to reduce the levels of serum triglycerides and low density lipoproteins (Leaf and Weber, 1988) and also stimulate the immune system (Wu and Meydani, 1996). As a result of its beneficial properties, Spirulina has long been produced as a supplement for human nutrition (Belay et al., 1996) but only recently it started to be used in animal diets and associated to improvements in animal growth, health, fertility as well as product quality. Hugh et al. (1985) reported that crossbred weanling pigs had up to 9% higher growth rates when received dietary Spirulina supplementation. Similarly Grinstead et al. (2000) found increased growth in pigs after completing their diet by 0.2%, 0.5% and 2% Spirulina. On the other hand, investigating the productive and meat quality in rabbits in response to Spirulina in the diet (at levels of 5, 10 and 15%), Peiretti and Meineri (2008, 2011) found no effect on production, however the lipid content and fatty acid profile of the meat were favorably changed.

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The aim of this work is to provide information on the lipid profile and fatty acid composition of *m. Longissimus dorsi* in pigs in response to dietary Spirulina supplementation.

Materials and Methods

Animals and diets

The experiment was carried out in the experimental farm of the Agricultural Institute - Shumen. A total of 33 Danube White pigs were randomly allocated in three groups (control and two experimental), each containing 11 animals. The experiment was divided in two sub periods, starting at 34 kg live weight and ending at 102.5-106.3 kg live weight. During the first sub period (30 kg – 60 kg live weight) the pigs from the control group were fed concentrate containing 17.15% crude protein, 0.84% lysine, 12.83 MJ metabolizable energy, 1.01% calcium and 0.69% phosphorus, and during the second sub period (60 kg - 110 kg live weight) the animals were fed compound feed containing 15.17% crude protein, 0.72% lysine, 12.76 MJ metabolizable energy, 0.52% calcium and 0.46% phosphorus. The pigs from the experimental groups were fed the same diet used for the control group. The first experimental group (SP1) received additionally 2 g *Spirulina platensis* per capita daily only during the second sub period, while the pigs from the second experimental group (SP2) received the same amount (2 g) of Spirulina during the whole trial period. The used supplement of *Spirulina platensis* for the study was produced by Platensis Ltd., Lithuania. Its chemical and fatty acid composition is presented in Table 1.

Slaughtering and sampling

At the end of the experiment the pigs were slaughtered at a live weight of 102.5-106.3 kg. After cold storage for 24 h at 4˚C, *m. Longissimus dorsi* was carefully dissected from the left side of 6 pig carcasses of each group. The muscle was cut into pieces and minced in meat grinder. Samples of 10 g were taken; vacuum packed and immediately stored at - 20˚C until extraction for analysis of the lipid profile and fatty acid composition.

Lipid analysis

Lipid and fatty acid analyses were carried out in the Laboratory of lipid analysis in the Department of Ecology and Quality of Animal Production, Institute of Animal Science-Kostinbrod. Total lipids were extracted from the muscles according to the method of Bligh and Dyer (1959). Aliquots of the lipid extracts were subjected to a phospholipids (PL) content assay, using the method of Bartlett (1959). For fatty acid determination, triacylglycerols (TG) and phospholipids were isolated by preparative thin layer chromatography. Methyl esters of the TG and PL were obtained using a 0.01% solution of sulfuric acid in dry methanol at 47°C for 14 h as described by Christie (1973). The fatty acid composition of the lipid fractions was analysed using gas chromatograph CSi 200 equipped with capillary column (TR-FAME - 60 m x 0.25 mm x 0.25 µm) and hydrogen as a carrier gas. The oven temperature was first set at 160°C for 0.2 min, and then raised until 220°C at a rate of 5°C/min and hold for 5 minutes. The temperatures of the detector and injector were 230°C. Methyl esters were identified comparing to the retention times of the standards. Fatty acids are presented as percentages of the total amount of the methyl esters identified (Christie, 1973).

Statistical analysis

Statistical analysis of the data was performed using JMP, v.7 statistical software. The effect of the dietary Spirulina supplementation was evaluated through one-way ANOVA. Post-hoc comparisons between groups were done by Fisher's LSD, as differences below 0.05 were considered significant.

Table 1
Chemical and fatty acid composition of *Spirulina platensis*

Item	Content, %
Water	15.67
Dry matter	84.33
Organic matter	70.37
Protein	40.27
Fat	0.061
Fibers	4.45
Minerals	13.96
Calcium	0.68
Non-nitrogen extract substances	25.59
C14:0 (Myristic)	0.45
C16:0 (Palmitic)	49.45
C16:1(Palmitoleic)	3.2
C18:0 (Stearic)	4.66
C18:1 (Oleic)	6.94
C18:2 (Linoleic)	17.25
C18:3n-6 (γ-linolenic)	17.23
C18:3 n-3 (α-linolenic)	0.82

Results and Discussion

Dietary Spirulina platensis supplementation did not induce significant changes in the content of the total lipids in the pigs (Table 2). However their amount was increased by 50% in the pigs in SP1 while that of the pigs in SP2 group was increased by 3.9% when compared to the control group. Contrary to our results Šimkus et al. (2013) reported slight but significant decrease in the intramuscular lipid content in *m. Longissimus dorsi* in pigs supplemented *Spirulina platensis* in amount 2 g/animal/d. On the other hand Peiretti and Meineri (2011) found increase in the lipid content in the Longissimus muscle in rabbits fed *Spirulina platensis*, which is in line with our results.

Algae supplementation had no effect on the PL and TG content in the Longissimus muscle of the pigs. The latter displayed higher content in the SP1 group when compared to the control and SP2 (by 67% and 7.69%, respectively), while the amount of phospholipids in the three groups remained relatively close within 0.35-0.40 g/100 g muscle. The values for the phospholipid content in this study are almost equal to those reported by Wood et al. (2004) in *m. Longissumus dorsi* of four breeds of pigs that varied between 0.38-0.42 g/100 g muscle. Phospholipids are important constituent of the cell membranes and their amount remains relatively constant or increases a little when the fatness of the animal increases (Wood et al., 2008). On the other hand the content of triacylglycerols strongly correlates to that of total lipids.

The major parts of the saturated fatty acids in TG of the muscles are C16:0 and C18:0. Their contents varied between 25.48 - 26.07% and 10.88 - 11.44% respectively (Table 3). The dietary Spirulina supplementation had no influence on the content of neither of them, only a slight tendency towards increase in the level of C18:0 was observed in the supplemented groups. *Spirulina platensis* in the diet influenced the content of C14:0 in Longissimus muscle. Its amounts were within the range of 1.63 - 1.80% and decreased in the pigs receiving the supplement, more substantially in the first

Table 2
Total lipids, triacylglycerols and phospholipids content in *m. Longissimus dorsi* in pigs receiving dietary *Spirulina platensis* supplementation

Item	C[1]	SP1[2]	SP2[3]
Total lipids [4]	1.28±0.14	1.92±0.49	1.33±0.22
Triacylglycerols[4]	0.91±0.14	1.52±0.49	0.98±0.23
Phospholipids[4]	0.37±0.01	0.40±0.03	0.35±0.03

Values are presented as Mean±SEM
[1]C - control group; [2]SP1 - group receiving Spirulina during the first sub period (60-110 kg); [3]SP2 - group receiving *Spirulina platensis* during the whole trial; [4] g/100 g muscle

Table 3
Fatty acid composition of triacylglycerols in *m. Longissimus dorsi* in pigs receiving dietary *Spirulina platensis* supplementation

Fatty acids, %	C[1]	SP1[2]	SP2[3]
C14:0 (Myristic)	1.80±0.036[a]	1.63±0.032[b]	1.67±0.063[ab]
C16:0 (Palmitic)	25.76±0.25	25.48±0.52	26.07±0.42
C17:0 (Margaric)	0.13±0.01	0.14±0.02	0.14±0.01
C18:0 (Stearic)	10.88±0.45	11.24±0.16	11.44±0.62
SFA[4]	38.57±0.65	38.49±0.54	39.32±1.03
C16:1 (Palmitoleic)	3.32±0.41	3.27±0.22	3.50±0.27
C18:1 (Oleic)	53.94±0.77	54.40±0.47	53.06±1.40
MUFA[5]	57.26±0.90	57.67±0.25	56.56±0.95
C18:2 (Linoleic)	3.49±0.31	3.18±0.18	3.44±0.16
C18:3n-6 (γ-linoleinic)	0.02±0.01	0.04±0.02	0.04±0.02
C18:3n-3 (α-linolenic)	0.17±0.01	0.16±0.01	0.17±0.01
C20:2 (Eicosadienoic)	0.20±0.01	0.18±0.01	0.19±0.02
C20:3 (Eicosatrienoic)	0.06±0.01	0.05±0.01	0.03±0.01
C20:4 (Arachidonic)	0.23±0.02	0.23±0.01	0.28±0.01
C20:5 (Eicosapentaenoic)	ND[7]	ND	ND
C22:5 (Docosapentaenoic)	ND	ND	ND
C22:6 (Docosahexaenoic)	ND	ND	ND
PUFA[6]	4.17±0.35	3.84±0.19	4.15±0.20

Values are presented as Mean ± SEM; Means connected with different letters are significantly different ($P<0.05$)
[1]C - control group; [2]SP1 - group receiving Spirulina platensis during the first subperiod (60-110 kg); [3]SP2 - group receiving Spirulina platensis during the whole trial, [4]Saturated fatty acids, [5]Monounsaturated fatty acids, [6] Polyunsaturated fatty acids, [7]Not detected

experimental group. It has been generally accepted that dietary saturated fat is associated with increased risk of coronary heart diseases.

However more recent epidemiological studies show that the saturated fat may have different effect (Pietinen et al., 1997; Mozzafarian et al., 2004; Xu et al., 2006) and the individual saturated fatty acids do not necessarily affect equally the risk of coronary heart diseases and the food sources of these fatty acids may also play a role. For example, a diet high in stearic acid (C18:0) does not raise serum cholesterol levels and even lowers LDL cholesterol when compared to diets enriched with palmitic (C16:0) or myristic acid (C14:0), (Grande et al., 1970; Tholstrup et al., 2004). The effect of *Spirulina platensis* in the diet on the content of C14:0 was not strongly demonstrated as to induce significant changes in the total amount of the saturated fatty acids and the content of this fatty acid remained similar in the three groups.

Oleic acid (C18:1) is the most abundant fatty acid in the TG fraction of the muscles. In this study its content was within 53.06 - 54.40%. It was not influenced by the dietary Spirulina supplementation. Similarly, the other monounsaturated fatty acid C16:1 as well as the total amount of monounsaturated fatty acids remained unchanged in the experimental groups.

Triacylglycerols have low amounts of polyunsaturated fatty acids. The highest content of all PUFA in the triacylglycerols belongs to C18:2 (3.18-3.49%). The rest of the PUFA remain below 1%. Although Spirulina has high content of C18:3 n-6 its amount in the TG of *m. Longissimus dorsi* was very low (0.02 - 0.04%). In the experimental groups it doubled the content of the control group; however the difference was not significant, due to considerable variation within the groups. Contrary to our studies, in rabbit adipose tissue consisting mainly of triacylglycerols, Peiretti and Meineri (2011) found significant increase in the content of C18:3n-6. No effect of the Spirulina supplementation was found on the content of the individual and total polyunsaturated fatty acids in the pigs in this study.

As in TG, the saturated fatty acids that present the highest part in PL fraction are C16:0 and C18:0 and their contents resembled those in the triacylglycerols. Neither of these fatty acids was influenced by the dietary Spirulina supplementation, although the content of C16:0 tended to decrease with the increase of the supplement levels. The content of C14:0 in phospholipids was low and varied between 0.14 - 0.18% in *m. Longissimus dorsi* of the pigs (Table 4). Contrary to TG its amounts were not influenced by Spirulina in the diet. No effect of the supplement was observed for the total amounts of SFA.

The content of C18:1 and C16:1 in phospholipids was lower than that of triacylglycerols (15.69 - 17.61%, 0.44 -

Table 4
Fatty acid composition of phospholipids in *m. Longissimus dorsi* in pigs receiving dietary Spirulina platensis supplementation

Fatty acids, %	C[1]	SP1[2]	SP2[3]
C14:0 (Myristic)	0.18±0.04	0.14±0.01	0.16±0.02
C16:0 (Palmitic)	22.30±0.84	19.98±0.46	18.99±0.90
C17:0 (Margaric)	0.48±0.03	0.44±0.03	0.45±0.02
C18:0 (Stearic)	11.01±0.31	10.98±0.38	11.03±0.61
SFA[4]	33.97±1.15	31.54±0.38	30.63±1.26
C16:1 (Palmitoleic)	0.47±0.13	0.44±0.05	0.64±0.05
C18:1 (Oleic)	15.69±1.20	16.48±0.61	17.61±1.01
MUFA[5]	16.16±1.31	16.92±0.64	18.25±0.99
C18:2 (Linoleic)	34.78±1.37	35.00±0.99	33.53±0.82
C18:3n-6 (γ-linoleinic)	$0.34±0.02^{a}$	$0.46±0.02^{b}$	$0.43±0.04^{ab}$
C18:3n-3 (α-linolenic)	0.37±0.04	0.45±0.04	0.45±0.04
C20:2 (Eicosadienoic)	0.61±0.02	0.81±0.08	0.74±0.07
C20:3 (Eicosatrienoic)	1.21±0.10	1.52±0.07	1.39±0.08
C20:4 (Arachidonic)	$11.37±0.60^{a}$	$12.00±0.36^{ab}$	$13.15±0.46^{b}$
C20:5 (Eicosapentaenoic)	$0.25±0.02^{a}$	$0.28±0.01^{a}$	$0.34±0.02^{b}$
C22:5 (Docosapentaenoic)	0.84±0.04	0.93±0.07	0.99±0.05
C22:6 (Docosahexaenoic)	0.09±0.01	0.08±0.02	0.10±0.01
PUFA[6]	49.86±1.76	51.53±0.69	51.12±1.01

Values are presented as Mean ± SEM; Means connected with different letters are significantly different ($P<0.05$)
[1]C - control group; [2]SP1 - group receiving Spirulina platensis during the first subperiod (60-110 kg); [3]SP2 - group receiving Spirulina platensis during the whole trial, [4]Saturated fatty acids, [5]Monounsaturated fatty acids, [6] Polyunsaturated fatty acids

0.64% respectively). No influence of Spirulina was detected on these fatty acids, however in the Longissimus muscle of the pigs receiving Spirulina for a longer period (SP2) the content of C16:1 was augmented by 36% and 45% when compared to the control and the experimental pigs of SP1. The changes in the total amount of MUFA followed the pattern of C18:1 whose content tended to be higher in the SP2 group than that of the control and the pigs of SP1. Despite these trends no significant influence of the Spirulina supplement was determined.

Phospholipid fraction is characterized by high content of polyunsaturated fatty acids, most abundant of which are C18:2 and C20:4. The content of C18:2 was within the range of 33.53 - 35.00%. It was not changed in response to the dietary Spirulina supplementation, while the content of C20:4 have been increased significantly ($P < 0.05$) the longer Spirulina was fed. Its amounts varied between 11.37 - 13.15% in the Longissimus muscle of the pigs in our study. In rabbits, Dal Bosco et al. (2014) found no significant difference between control and Spirulina supplemented group in terms of the content of C20:4, however they reported significant increase in C18:2.

The contents of C18:3n-6 was within the range of 0.34 - 0.46% indicating that this fatty acid is incorporated in higher amounts in the phospholipid fraction than in TG. It was significantly ($P < 0.05$) influenced by the dietary Spirulina supplementation, as its amounts were at average 31% higher than that of the control group. Such augmentation was reported by Peiretti and Meinery (2011) and Dal Bosco et al. (2014) in rabbits.

The contents of C18:3n-3 showed trend toward increase with increasing of Spirulina supplementation in the diet and was augmented by 21.6% for the pigs in SP1 and SP2 groups. The rest of the n-3 polyunsaturated fatty acids – C20:5 and C22:5 followed the same pattern of changes as C18:3. The content of C20:5 was influenced significantly ($P < 0.05$) by the dietary Spirulina supplementation, whereas the amount of C22:5 was increased by the average 14% when compared to the control group. Our results are not in agreement with those of Dal Bosco et al. (2014), who indicated significant decrease in the n-3 PUFA in *m. Longissimus dorsi* in rabbits. Despite the observed changes in the content of the individual polyunsaturated fatty acids, no such results were found for the total amount of PUFA.

Conclusion

The results of this study show certain positive effect of the dietary Spirulina supplementation in amount 2 g/d/animal on the lipid profile and fatty acid composition in *m. Longissimus* dorsi in pigs. As a result of Spirulina in the diet significant decrease in the content of the hypercholesterolemic C14:0 was observed in triacylglycerol fraction. On the other hand phospholipids displayed higher amounts on C18:3n-6, C20:4, C20:5 as well as tendencies toward higher amount of C18:3n-3 and C22:5 in the supplemented groups. These polyunsaturated fatty acids play important role in the organism and their increase especially of n-3 PUFA is beneficial for the good nutritional and healthy meat characteristics.

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