Bulgarian Journal of Agricultural Science, 19 (No 6) 2013, 1416-1424
Agricultural Academy

EFFECT OF FLAXSEED AND ALPHA TOCOPHEROL SUPPLEMENTATION OF PIG DIETS ON FATTY ACID CONTENT AND LIPID OXIDATION STABILITY OF *M. LONGISSIMUS*

V. DOYCHEV* and G. MIHAYLOVA
Trakia University, Faculty of Agriculture, BG - 6000 Stara Zagora, Bulgaria

Abstract

DOYCHEV, V. and G. MIHAYLOVA, 2013. Effect of flaxseed and alpha tocopherol supplementation of pig diets on fatty acid content and lipid oxidation stability of *m. Longissimus. Bulg. J. Agric. Sci.,* 19: 1416-1424

The experiment was carried out with 70 fattening hybrid pigs (45-110 kg) divided into 5 groups of 14 (7 castrated males and 7 females). Pigs were fed compound feed containing 13.24% CP and 12.04 MJ ME/кg. During the last two months of the fattening period (78–120 kg) the compound feed of pigs from groups II, III, IV and V (experimental) was supplemented with flaxseed at different levels: group II – 4%, group III – 8%; groups IV and V – 12%. The feed of pigs from group V was also supplemented with vitamin E (alpha tocopherol acetate) at 200 mg/kg. Pigs were slaughtered at 120 kg live body weight. The fatty acid contents of triacylglycerols and phospholipids were determined in m. Longissimus samples. Oxidative changes in m. Longissimus meat were assessed in fresh samples and in samples stored at –18°C for 6 months.

Linolenic acid content of triacylglycerols increased in experimental groups, being statistically significant higher in group II ($P<0.01$), III, IV and V ($P<0.001$) vs group I. Linoleic acid concentrations also differed between groups II and III ($P<0.05$), group II and both groups IV and V ($P<0.001$) and groups III and IV ($P<0.01$). Compared to control pigs, eicosapentaenoic acid content in tissue triacylglycerols was higher in group III ($P<0.05$), IV and V ($P<0.01$). Significant differences were also established in groups IV and V vs group II ($P<0.05$). Linoleic acid content decreased in group III vs group I ($P<0.05$) and group II ($P<0.01$), whereas arachidonic acid changed only slightly. The PUFA content was also insignificantly altered. Compared to the non-supplemented group, the PUFA/SFA ratio was substantially higher in groups II, IV and V ($P<0.01$) and III ($P<0.05$). The n-6/n-3 ratio in groups III, IV and V was significantly lower than that of groups I and II ($P<0.001$). The difference between groups I and II was also considerable ($P<0.001$).

Linolenic acid in tissue phospholipids of group III was significantly more than that in groups I ($P<0.01$) and II ($P<0.05$). In groups IV and V, the content f this fatty acid was higher than that in groups I, II ($P<0.001$) and III ($P<0.01$). The eicosapentaenoic acid in groups IV and V was considerably higher than that in control ($P<0.05$). There were no statistically significant differences between groups with regard to fatty acid groups. The n-6/n-3 ratio was lower in group II vs group I ($P<0.05$) and in groups III, IV and V ($P<0.001$) vs group I. The differences between groups IV and V vs group II were also important ($P<0.01$).

The high content of PUFA in tissue lipids resulted in significantly higher thiobarbituric acid number in both fresh and stored meat.

Key words: pigs, flaxseed, fatty acid composition, triacylglycerols, phospholipids, vitamin E, oxidation

*Abbreviations:*MDA – malondialdehyde; MUFA– monounsaturated fatty acids; SFA – saturated fatty acids; UFA – unsaturated fatty acids; ME – metabolizable energy; PUFA – polyunsaturated fatty acids; CP – crude protein; TBARS – thiobarbituric acid reactive substances; n-3 – omega-3; n-6 – omega-6.

Introduction

Polyunsaturated fatty acids (PUFA) of the omega-3 group are beneficial for human health (Narayan et al., 2006), and that is why dietologists have long worked to increase their content in man's diet. The easiest way to increase dietary PUFA intake is by higher consumption of fish. Unfortunately, in many countries including Bulgaria, fish is not among traditional foods, which necessitates seeking how to increase PUFA content of common animal foodstuffs (meat, eggs

E-mail: v.doichev@gmail.com

etc.). Pigs, being monogastric animals (with a simple, one-compartment stomach) are suitable for production of PUFA enriched meat. The fatty acids from feed are absorbed and directly included in lipid synthesis unchanged, unlike in ruminant forestomach, where PUFA are hydrogenated.

With regard to the fatty acid content and oxidation stability of lipids, flaxseed is though to be the best choice for enrichment of pork with n-3 fatty acids, being superior to rapeseed and oils obtained from flaxseed, rapeseed and fish (Van Oeckel et al., 1995). The problems arising from the use of flaxseed in feed manufacturing are related to the presence of some anti-nutritional factors (Liener, 1980; Batterham et al., 1991; Niedwietz-Siegen, 1998), high mucilase content (Batterham et al., 1994), and the higher costs compared to traditional cereal crops.

Another serious problem from pork enrichment with n-3 fatty acids is the reduction of lipids stability to oxidation and hence, worsening of the flavour of meat and meat products. Many researchers believe that the enrichment of muscle lipids with PUFA makes meat more susceptible to oxidation and reduces the storage terms (Leskanich et al., 1997; Wood et al., 2003; Guo et al., 2006). This problem could be overcome by inclusion of vitamin E in the diet, a natural antioxidant that accumulates in tissues, largely prevents oxidation and improves the quality of meat and meat products both in fresh state and after storage (Dikeman, 2007; Santos et al., 2008; Trefan et al., 2011). On the contrary, others did not establish a higher antioxidant status of muscles after supplementation of the ration with vitamin E (Lauridsen et al., 1999; Shaw et al., 2002).

The purpose of this experiment was to determine the effect of different dietary flaxseed levels upon the fatty acid content and oxidation stability of meat lipids and to evaluate the efficacy of alpha tocopherol for reduction of oxidative changes in tissue lipids with high PUFA content.

Material and Methods

The experiment was carried out with 70 fattening hybrid pigs crosses of Swedish Large White (SLW) × Bulgarian Landrace (BL) × Hybrid ham boars (HB), divided into 5 groups of 14 (7 castrated males and 7 females). Pigs were fed compound feed containing 13.24% CP and 12.04 MJ ME/кg. During the last two months of the fattening period (78–120 kg) the compound feed of pigs from groups II, III, IV and V (experimental) was supplemented with flaxseed at 40, 80, 120 and 120 $g.kg^{-1}$, respectively. The feed of pigs from group V was also supplemented with alpha tocopherol acetate at 0.2 $g.kg^{-1}$. The composition and the nutritional value of compound feed for the five groups of pigs is presented in Table 1.

Table 1
Composition and nutritional value of compound pig feed ($g.kg^{-1}$)

Ingredients	Groups				
	I	II	III	IV	V
Corn	80	53.4	26.8	–	–
Barley	328	430.6	534	638.2	638.2
Wheat	310	257	203.2	149.8	149.8
Flaxseed	–	40	80	120	120
Soybean meal	30	20	10	–	–
Wheat bran	160	107	54	–	–
Provimi concentrate	50	50	50	50	50
Zeolite	35	35	35	35	35
Synthetic lysine	2.4	2.4	2.4	2.4	2.4
Limestone	2.6	2.6	2.6	2.6	2.6
Toxibind	2	2	2	2	2
α–tocopherol	–	–	–	–	0.2
One kg feed contains:					
Metabolizable energy, MJ	12.23	12.39	12.56	12.72	12.68
Crude protein, g	132.4	133	133.6	134.2	134.1
Crude fibre, g	45	44.8	44.7	44.5	44.6
Lysine, g	7.7	7.65	7.56	7.47	7.49
Methionine+cysteine, g	5.1	4.96	4.8	4.64	4.66
Ca, g	6,29	6.3	6.3	6.33	6.3
P, g	4,65	4,4	4.1	3.83	3.85

The fatty acid composition of feed is given in Table 2. Fattening pigs were slaughtered at 120 kg live body weight.

For fatty acid composition of meat lipids, samples were collected from m. Longissimus thoracis (in the area of the last three thoracic vertebrae).

Meat lipids were extracted by the method of Bligh and Dyer (1959). Lipid groups – triacylglycerols and phospholipids were isolated by thin-layer chromatography on silica gel G plates and mobile phase consisting of hexane/ether (80:20) as per Dimov and Dimitrov (1978). Fatty acids were methylated in 2% H_2SO_4 dissolved in anhydrous methanol according to the technique of Angelov (1994). The fatty acid composition of total lipids was determined on a gas chromatograph "Payunicam" with ECTM-WAX capillary column 30 m, ID 0.25 mm, and flame ionisation detection.

Thiobarbituric acid reactive substances (TBARS) were determined as per Ohkawa et al. (1979), modification of Pikul et al. (1983). TBARS was expressed as mg MDA (malondialdehyde) /g fat. MDA content was calculated from sample absorptions against a standard curve (0-5.5 mg).

The results were processed with statistical software Statistica for Windows, Release, 4.3 (Stat. Soft. Inc., 1994).

Results and Discussion

The fatty acid composition of triacylglycerols in m. longissimus is presented in Table 3. There were statistically significant changes in the three SFA between groups. Myristic acid content was substantially lower in group II ($P<0.01$) and groups III, IV and V ($P<0.05$) compared to controls. Palmitic acid content decreased proportionally to increased dietary flaxseed level and was significantly lower in all supplemented groups ($P<0.01$) compared to group I. The higher the flaxseed content of feed, the lower stearic acid concentrations

Table 2
Fatty acid composition of compound pig feed

Fatty acids (g/100 g fat)	Flaxseed content in feed, g/kg				
	0	40	80	120	120
C14:0 Myristic	0.7	1	0.8	0.9	0.9
C16:0 Palmitic	29.3	31.1	25.7	26.8	27.2
C16:1 Palmitoleic	1.8	1.4	1.3	1.2	1.4
C17:0 Margaric	0.8	0.6	1.2	1.3	1.1
C18:0 Stearic	12.1	11.6	10.8	9.8	9.6
C18:1 Oleic	35.4	35.3	36.1	36.8	36.3
C18:2 Linoleic	19.4	16.7	18.5	15.6	15.9
C18:3 Linolenic	-	2	5.4	7.2	7.2

Table 3
Fatty acid composition of triacylglycerols in m. longissimus

Fatty acids (g/100 g fat)	Groups				
	I	II	III	IV	V
	X ±Sx	X ±Sx	X ±Sx	X ±Sx	X ±Sx
C14:0 Myristic	2.70^{a}±0.84	1.35^{b}±0.52	1.73^{b}±0.64	1.52^{b}±0.43	1.58^{b}±0.48
C16:0 Palmitic	26.46^{a}±1.63	24.52^{b}±0.50	24.24^{b}±0.53	23.84^{b}±0.71	24.01^{b}±0.83
C16:1 Palmitoleic	4.67^{a}±1.13	3.69^{ac}±1.16	1.36^{b}±0.49	2.40^{bc}±0.43	2.23^{bc}±0.64
C18:0 Stearic	15.54^{a}±1.13	15.00^{ac}±0.84	14.05^{bc}±0.47	13.79^{b}±0.19	13.93^{b}±0.28
C18:1 Oleic	42.32^{a}±1.27	46.34^{b}±1.16	50.12^{c}±0.95	49.38^{cd}±0.52	48.93^{cd}±0.86
C18:2 Linoleic	6.98^{a}±0.60	7.21^{a}±0.39	6.24^{b}±0.42	6.52^{ab}±0.47	6.55^{ab}±0.68
C18:3 Linolenic	0.55^{a}±0.08	1.14^{b}±0.06	1.59^{c}±0.07	1.85^{cd}±0.13	1.87^{cd}±0.14
C20:4 Arachidonic	0.70±0.51	0.51±0.27	0.52±0.10	0.48±0.14	0.50±0.12
C20:5 Eicosapentaenoic	0.08^{a}±0.01	0.15^{ab}±0.05	0.15^{bd}±0.04	0.22^{cd}±0.08	0.21^{cd}±0.06
PUFA	8.31±0.76	9.01±0.62	8.50±0.50	9.07±0.48	9.11±0.72
PUFA/SFA	0.19^{a}±0.02	0.22^{b}±0.01	0.21^{b}±0.01	0.23^{b}±0.01	0.23^{b}±0.02
n-6/n-3 ratio	12.15^{a}±0.82	5.99^{b}±0.31	3.88^{c}±0.20	3.39^{c}±0.09	3.39^{c}±0.12

Different superscripts indicate statistically significant differences of mean values

were measured – they were statistically significantly lower in group III ($P<0.05$) and groups IV and V групи ($P<0.01$) vs group I. Significant differences were also established in groups II and IV and V ($P<0.05$). Our results were similar to those of Riley et al. (2000) и Kouba et al. (2003), demonstrating a significantly lower saturated fatty acid content after supplementing pig feed with flaxseed.

Palmitoleic acid concentrations were lower in supplemented groups, with statistically significant difference in group III ($P<0.001$) and groups IV and V ($P<0.01$) vs group I. There was also a considerable difference in palmitoleic acid content between groups II and III ($P<0.01$). Unlike palmitoleic acid, the oleic acid level increased significantly and parallelly to the dietary flaxseed content in all supplemented groups ($P<0.001$) vs the controls. Oleic acid content in groups III, IV and V was also higher than that of group II ($P<0.001$). The available results about the effect of PUFA-enriched feeds on tissue MUFA concentrations in pigs are contradictory. After addition of 3.5% blend of tallow and soybean oil (80%:20%) and 6% flaxseed in the feed of control group, Kouba et al. (2003) established reduction in both palmitoleic and oleic acid contents. The supplementation of pig feed with 15% flaxseed by Specht-Overholt et al. (1997) resulted in lower oleic acid content, although palmitoleic acid concentration remained unchanged. Riley et al. (2000) did not observe any statistically significant differences in monounsaturated fatty acids. According to Duran-Montge et al. (2008) MUFA were more susceptible to alteration compared to SFA when the ration of pig was supplemented with PUFA. Probably, the mechanism of flaxseed effect on the concentrations of the different MUFA needs further detailed investigation.

Linolenic acid exhibited more than threefold increase with statistically significant differences vs group I in groups II ($P<0.01$), III, IV and V ($P<0.001$). There were also substantial differences between groups II and III ($P<0.05$) and group II and each of groups IV and V ($P<0.01$). The level of eicosapentaenoic acid, a derivative of linolenic, was almost three times higher in pigs fed with increasing feed flaxseed content. Its concentrations were statistically significantly higher in group III ($P<0.05$) than in group I, in groups IV and V compared both to group I ($P<0.01$) and group II ($P<0.05$). Due to the higher linolenic acid content of flaxseed – about 50% of lipid content, many authors have conducted experiments with different dietary levels of flaxseed (Cherian and Sim, 1995; Romans et al., 1995; Van Oeckel et al., 1996; Leskanich et al., 1997; Enser et al., 2000; Riley et al., 2000; Kouba et al., 2003; Juarez et al., 2010; Kouba and Mourot, 2011; Nijten et al., 2011), established an important increase in tissue linolenic acid and its derivative, eicosapentaenoic acid.

The changes in fatty acids from the n-6 group were less pronounced compared to n-3 fatty acids. Linoleic acid decreased when dietary flaxseed content was higher. In group III, its value was significantly lower compared to group I ($P<0.05$) and II ($P<0.01$). Arachidonic acid, a derivative of linoleic, also decreased in a similar manner but the between-group variations were insignificant ($P>0.05$). Our results were comparable to those of Cherian and Sim (1995) having supplemented pig feeds with flaxseed.

The content of the PUFA group did not change considerably ($P>0.05$), as also outlined by Riley et al. (2000), who did not establish increased PUFA content after supplementation of the ration with 11.4% flaxseed. The addition of rapeseed and fish oils to pig feeds neither resulted in change in PUFA of meat (Leskanich et al. 1997). A possible explanation for the lack of PUFA content in the present experiment and data reported by others could be the thesis of Sprecher et al. (1995) that enzymes, responsible for the elongation and dehydration of linoleic and linolenic acid chains for synthesis of derivatives with longer chains, are concurrently used by n-6 and n-3 fatty acids. When pig feeds are supplemented with linolenic acid through flaxseed, these enzyme systems are mainly used for synthesis of n-3 fatty acids, because of the limited n-6 fatty acid synthesis. This results in altered n-6/n-3 ratio and in lower increase in PUFA level in general. In the view of Riley et al. (2000), feed supplementation with flaxseed increases linolenic content in tissue lipids allowing for a successful competition with linoleic acid to shift the biochemical metabolic pathways towards synthesis of n-3 fatty acids derivates with longer carbon chains.

The PUFA/SFA ratio of 0.19-0.23 was below the recommended minimum ratio of 0.40 (Department of Health and Social Security 1984; 1994). At the same time, it was higher in pigs receiving feed supplemented with flaxseed, with statistically significant differences between groups II, IV, V ($P<0.01$) and III ($P<0.05$) compared to group I. In their experiments with considerably lower flaxseed feed content (2.6%), Enser et al. (2000) also reported a certain but insignificant increase in this ratio. Warnants et al. (1999) used soybeans as a source of PUFA and succeeded to increase PUFA/SFA ratio from 0.16 to 0.23. Riley et al. (2000) increased the PUFA/SFA ratio by supplementing the feed with 11.4% flaxseed for a 24-day period. At the same time, the longer supplementation (65 days) with lower flaxseed levels (1%, 2% and 3%). did not result in higher ratios. The ratios in both our results and the data of cited researchers were low, under the recommended allowance of 0.4 for human food. A probably explanation is proposed by Raes et al. (2004), i.e. that feed components rich in n-3 PUFA (fish products, vegetable oils and oilseeds) did not have a strong effect on PUFA/SFA ratio, as the latter was

mainly determined by the genetic factor and at a lesser extent, by the nutrition.

With increasing dietary flaxseed content, the n-6/n-3 ratio decreased to attain statistically significantly lower values in groups III, IV and V compared to both groups I and II ($P<0.001$). The difference between groups I and II was also important ($P<0.001$). The observed reduction in this ratio corresponds to numerous published results. Riley et al. (2000) reported a considerably reduced n-6/n-3 ratio in both the short-term feeding trial with high dietary flaxseed content (11.4%) and in the trial with feeding dietary flaxseed levels 1%, 2% and 3% over 65 days. The inclusion of 10%, 17.5% and 25% flaxseed in pig feeds resulted in considerable decrease in n-ratio from 8.8 to 1.0 (Cherian and Sim, 1995). Leskanich et al. (1997) established reduction of the ratio from 7.3 in the control group to 4.5 in experimental groups after supplementing pig feed with 2% rapeseed oil and 1% fish oil. The addition of 6% flaxseed in pigs' feed led to lower N-ratios: 2.45 times after 20 days, 2.98 times after 60 days and 3 times – after 100 days (Kouba et al., 2003).

It could be therefore concluded that the dietary supplementation with flaxseed resulted to significant changes in the fatty acid content of triacylglycerols of porcine m. longissimus. The n-3 fatty acids increased substantially, while n-6 fatty acids content was either unchanged or slightly lower. This entails a statistically significant reduction of the n-6/n-3 ratio from 12.15 in control pigs to 5.99 (group II), 3.88 (group III), 3.39 (groups IV and V). The n-6/n-3 values in group III, which received feed with 8% flaxseed and in groups IV and V, fed a ratio with 12% flaxseed, were compliant to the nutritional recommendations of dietologists for consumption of foodstuffs with n-6/n-3 ratio below 5, whereas in group II, supplemented with 4% flaxseed, this ratio was >5.

The PUFA/SFA ratio, another dietetic criterion of meat, was not significantly improved. Although this ratio in experimental groups was statistically significantly higher compared to controls, the increase is not important. Except for the maximum values in groups IV and V – 0.23 – it remained rather under the level of 0.4, recommended by dietologists.

The fatty acid composition of m. longissimus phospholipids is presented in Table 4. Changes in SFA occurred only in palmitic acid content, which decreased when dietary flaxseed percentage increased. Palmitic acid concentrations were statistically significantly lower in groups IV and V compared to groups I and II ($P<0.05$). The other two SFA – myristic and stearic, did not change ($P>0.05$). Our results did not completely correspond to those of other authors' experiments with addition of flaxseed to pig diets. Some reported that SFA content was unaltered (Romans et al., 1995; Specht-Overholt et al., 1997), and other demonstrated increased stearic acid concentrations without changes in myristic and palmitic acid contents in polar lipids of m. longisimus (Kouba et al., 2003). The inconsistency of data from the different researchers was probably due to the various experimental designs used. In the belief of Halenstvedt et al., (2012) both the content and the composition of dietary lipids should be took into consideration, as when the ration is supplemented with fat containing n-3 fatty acids, the latter are effectively deposited in tissue whereas the low fat ration provokes the synthesis of short-chain fatty acids.

Table 4
Fatty acid composition of phospholipids in m. longissimus

Fatty acids (g/100 g fat)	Groups				
	I	II	III	IV	V
	X ±Sx	X ±Sx	X ±Sx	X ±Sx	X ±Sx
C14:0 Myristic	1.32±0.67	1.83±0.88	1.81±0.13	1.82±0.45	1.83±0.64
C16:0 Palmitic	26.44^{a}±0.96	26.48^{a}±0.18	24.83^{ab}±0.41	24.22^{b}±0.63	24.78^{b}±0.84
C16:1 Palmitoleic	2.68±0.67	2.59±0.17	2.71±0.39	2.39±0.55	2.43±0.58
C18:0 Stearic	12.36±1.02	11.05±0.34	12.85±1.02	13.82±0.85	13.02±1.63
C18:1 Oleic	17.19±0.08	17.79±0.08	18.03±0.02	17.70±0.50	17.23±0.24
C18:2 Linoleic	31.11±1.12	31.04±0.85	29.75±1.48	30.28±1.47	30.80±1.26
C18:3 Linolenic	0.86^{a}±0.04	0.94^{a}±0.11	1.31^{b}±0.09	1.74^{c}±0.07	1.68^{c}±0.10
C20:4 Arachidonic	6.95^{a}±0.89	6.69^{ab}±0.16	6.79^{a}±0.07	5.85^{b}±0.11	5.97^{b}±0.24
C20:5 Eicosapentaenoic	1.08^{a}±0.18	1.60^{ab}±0.34	1.92^{ab}±0.35	2.17^{b}±0.49	2.24^{b}±.21
PUFA	40.00±0.01	40.26±1.51	39.78±1.67	40.05±2.11	40.69±1.76
PUFA/SFA	1.00±0.02	1.02±0.06	1.01±0.08	1.00±0.09	1.03±0.12
n-6/n-3 ratio	20.04^{a}±2.39	15.09^{c}±2.44	11.31^{bc}±0.45	9.22^{b}±0.92	9.38^{b}±1.18

Different superscripts indicate statistically significant differences of mean values

The content of MUFA (palmitoleic and oleic) in tissue phospholipids did not change after feed supplementation with flaxseed (P>0.05).

Linoleic acid remained unchanged along with dietary flaxseed content increase, and its derivative, arachidonic acid was reduced. Its levels in groups IV and V were statistically significantly lower as compared to groups I and III (P<0.05). In this respect our results are comparable to others, having accounted for no changes in linoleic acid together with decrease in arachidonic acid content (Cherian and Sim, 1995; Romans et al., 1995; Specht-Overholt et al., 1997; Kouba et al., 2003). Based on results obtained, Cherian and Sim (1995) suggested a particular type of interaction between α-linolenic and arachidonic acids. They assumed that the decreased arachidonic acid could indicate a competitive inhibition of its synthesis when feeds and tissues are richer in α-linolenic acid. This hypothesis is in accordance with the schedule proposed by Spresher et al. (1995) for enzyme systems competing in the synthesis of long-chain n-6 and n-3 fatty acids, already mentioned in discussing the results of fatty acid composition of m. longissimus triacylglycerols.

Linolenic acid content increased together with the dietary flaxseed content, and was statistically significantly higher in group III than in groups I (P<0.01) and II (P<0.05). In groups IV and V, linolenic acid concentrations were higher vs groups I, II (P<0.001) and III (P<0.01). Eicosapentaenoic acid levels also increased, being substantially higher in groups IV and V (P<0.05) than in controls. Our results corresponded to numerous data about elevated linolenic and eicosapentaenoic acids in meat phospholipids after addition of feed components rich in n-3 fatty acids in pig rations (Romans et al., 1995; Specht-Overholt et al., 1997; Enser et al., 2000; Kouba et al., 2003; Rey et al., 2004; Tikk et al., 2007; Lu et al., 2008; Halenstvedt et al., 2012).

The results made clear that PUFA distribution in the two lipid classes was different. Linoleic acid in phospholipids (Table 4) was 29.75 g/100 g fat (31.11%), and in triacylglycerols (Table 3) – 6.24 g/100 g fat (7.21%). At the same time, the distribution of linolenic acid was far more regular – 0.86 (1.74%) and 0.55 g/100 g fat (1.87%) in phospholipids and triacylglycerols, respectively. Long-chain PUFA – arachidonic and eicosapentaenoic, prevailed in the phospholipid fraction. Kouba et al. (2003) reported a comparable pattern of distribution of long-chain PUFA (arachidonic and eicosapentaenoic) in both lipid classes.

The PUFA group content was not altered, as also communicated by Spesht-Overholt et al. (1997) and Kouba et al. (2003).

The changes in PUFA/SFA ratios were not important, confirming the data of Enser et al. (2000) and Kouba et al. (2003). The ratios cited by these authors were similar to ours, respectively 0.97–1.07 (Enser et al., 2000) and 1.25–1.32 (Kouba et al., 2003).

The n-6/n-3 ratio was reduced parallelly to increasing flaxseed content of feeds, with lower values in group II (P<0.05), III, IV and V (P<0.001) vs group I. The difference between group II and groups IV and V гупа were also significant (P<0.01). Our results were similar to those reported in other experiments with flaxseed-supplemented pig diets (Specht-Overholt et al., 1997; Hoegberg et al., 2003; Kouba et al., 2003; Rey et al., 2004).

The lack of substantial changes in SFA, MUFA and some PUFA corresponds to the opinion of many researchers about the more conservative character of phospholipids compared to triacylglycerols. Cherian and Sim (1995) and Warnants et al. (1999) observed more pronounced changes in muscle tissue triacylglycerols than in phospholipids.

The more conservative character of phospholipids could be also seen from the slower magnitude of n-6/n-3 ratio changes – 2.17 times. For triacylglycerols, this ratio decreased 3.58 times. The high n-6/n-3 ratio in phospholipids observed in the present experiment was compliant with the results of others (Enser et al., 2000; De Smet et al., 2004), which attributed it to the normally high n-6 fatty acids content in phospholipids and their important structural role for cellular and subcellular membranes. The interpretation of the higher n-6/n-3 ratio and the lower observed fold change of this ratio in phospholipid, should account for the small share of phospholipids in the total lipids of muscles, hence their lesser effect on fatty acid composition and dietary properties of muscle lipids. Phospholipids, according to De Smet et al. (2004) comprise 22 to 26% of total muscle lipids.

The oxidative changes in m. Longissimus are presented in Table 5. Thiobarbituric acid reactive substances (TBARS) increased significantly in fresh meat, with statistically signifi-

Table 5
Oxidation changes in m. Longissimus, expressed through TBA numbers

Signs	Groups				
	I	II	III	IV	V
	X±Sx	X±Sx	X±Sx	X±Sx	X±Sx
Fresh state	0.08^{a}±0.02	0.09^{abc}±0.02	0.12^{bcf}±0.02	0.15^{def}±0.2	0.16^{def}±0.07
After storage	0.11^{abc}±0.03	0.12^{bcf}±0.03	0.13^{cdf}±0.04	0.15^{def}±0.04	0.12^{bcf}±0.03

Different superscripts indicate statistically significant differences in mean values

cantly higher values in group III (P<0.05), IV and V (P<0.001) compared to group I. The TBARS of groups IV and V were also considerably higher than those of group II (P<0.001). There were also significant differences between groups III and IV (P<0.05) and groups III and V (P<0.01). After storage, TBARS increased parallelly to PUFA content of tissues to attain a higher value in group IV vs group I (P<0.05). These data are in compliance to many reports stating higher level of oxidation changes in meat after enrichment of tissues with PUFA (Romans et al., 1995; Overland at al., 1996; Leskanich et al., 1997; Wood et al., 2003). Asghar et al. (1991) suggests that the high UFA content is among the primary pro-oxidation factors. Wood et al. (2003) reported that the enrichment of muscle lipids with PUFA rendered meat more sensitive to oxidation and reduced its shelf life. Contrary to cited opinions, other researchers did not observed any effect of dietary lipid supplements and the duration of their intake on oxidative changes in muscle lipids (Haak et al., 2006; Haak et al., 2008).

Significant differences in TBARS before and after storage were not observed in any of groups, meaning that the 6-month storage did not lead to significant oxidative changes. This finding was opposite to data of numerous authors, reporting increased TBA numbers of meat after storage (Monahan et al., 1990; Cannon et al., 1996; Hoving-Bolink et al., 1998; Corino et al., 1999; Waylan et al., 2002), which could be attributed to the different experimental designs. In our trials, the samples were stored frozen at –18°C for 6 months. It seems that very low temperatures minimised the oxidation processes despite the long storage period. In the experiments of cited authors, the samples have been held at 5–7°C in a refrigerator, so these higher temperatures could trigger more significant oxidative events although within a shorter period: from 2 to 10 days.

The data presented in Table 5, showed also an interaction of both studied factors. TBARS were higher in stored meat from groups II (P<0.05), III (P<0.05), IV and V (P<0.001) compared to fresh meat from the control group. Significant differences were also observed between values of fresh meat from group II and stored meat from group IV (P<0.01).

The significant difference between group I (after storage) and fresh meat from groups IV and V could, in our view, indicate that the PUFA tissue content was more important for occurrence of oxidative events than the term of storage.

No significant effect of dietary vitamin E on tissue oxidation could be demonstrated. In fresh meat, TBARS were even slightly higher in pigs from group V, whose feed was supplemented with 200 mg/kg. After storage, the TBARS in group V was insignificantly lower compared to that of group IV (P>0.05). This is in contradiction with the observed beneficial effect of tocopherol on pork lipids' stability in most of reported data (Monahan et al., 1992; Kim et al., 1994; Van Oeckel et al., 1995; Ahn et al., 2000; Guo et al., 2006; Dikeman, 2007; Santos et al., 2008; Trefan et al., 2011). Unlike the cited authors, Hertzman et al. (1988) did not establish a favourable effect of vitamin E in feed on oxidation of tissue lipids, whereas in the view of Lauridsen et al. (1999), vitamin E added to feed did not alter the antioxidant stratus of pig muscles.

Conclusions

The supplementation of pig feeds with flaxseed resulted in considerable changes in the fatty acid composition of triacylglycerols of m. longissimus. The content of n-3 fatty acids increased statistically significantly whereas the content of n-6 fatty acids was either unchanged or slightly reduced. The n-6-/n-3 ratio was also significantly altered. The PUFA/SFA ratio although significantly increased, remained under the recommended dietary allowance of 0.4. The phospholipids of m. Longissimus exhibited a more conservative pattern of change and hence, slighter changes in fatty acid composition consequently to dietary changes. The distribution of linolenic acid (C18:3) in the two lipid classes (triacylglycerols and phospholipids) was more regular than that of linoleic acid (C18:2). Tissue lipids PUFA content appeared to be more important for the development of oxidative events than the duration of storage. No inhibiting effect of dietary vitamin E supplementation with respect to meat oxidation was found out.

References

Angelov, A., 1994. Effect of dietary vegetable oils intake upon tissue lipids composition and content in early-weaned lambs. PhD Thesis, pp. 47-48 (Bg).

Dimov, V. and G. Dimitrov, 1978. Investigation of blood non-esterified fatty acids. *Animal Science*, **XV** (4): 92-98 (Bg).

Ahn, D. U., C. Jo. M. Du, D. G. Olson and K. C. Nam, 2000. Quality characteristics of pork patties irradiated and stored in different packaging and storage conditions. *Meat Science*, **56**: 203–209.

Asghar,A., J.I .Gray, A. M.Booren, E. A.Gomaa, M. M. Abouzied, E. R. Miler and D. J. Buckley, 1991. Effect of supranutritional dietary Vit. E levels on subcelular deposition of α - tokopherol in the muscle and on pork quality. *J. Sci. Food Agric.,* **57**: 31-41.

Batterham, E. S., L. M. Andersen, D. R. Baigent and A. G. Green, 1991. Evaluation of meals from Linola TM low linolenic acid linseed and conventional linseed as protein sources of growing pigs. *Anim. Feed Sci. and Technol*., **35**: 181-190.

Batterham, E. S., L. M. Andersen and A. G. Green, 1994. Piridoxin supplementation of Linola TM meal for growing pigs. *Anim. Feed Sci. and Technol*., **50**: 167-174.

Bligh, E. G. and W. J. Dyer, 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37** (8): 911-917

Cannon, J. E., J. B. Morgam, G. R. Schmidt, J. D. Tatum, J. N. Sofos, G. C. Smith, R. G. Delmore and S. N. Williams, 1996. Growth and fresh meat quality characteristics of pigs supplemented with Vit. E. *J. Anim. Sci*., **74**: 98-105.

Cherian, G. and S. Sim, 1995. Dietary alfha-linolenic acid alters the fatty acid composition of lipid classes in swine tissues. *Journal of Agricultural and Food Chemistry*; **43**: 2911-2916.

Corino, C., G. Oriani, L. Pantaleo, G. Pastorelli and G. Salvatori, 1999. Influence of dietary vitamin E supplementation on heavy pig carcass characteristics, meat quality, and vit. E status. *J. Anim. Sci*., **77**: 1755-1761.

Department of Health and Social Security, 1984. Report on Health and Social Subjects No. 28. Nutritional Aspects of Cardiovascular and Disease. London. HMSO.

Department of Health and Social Security, 1994. Report on Health and Social Subjects No. 46. Nutritional Aspects of Cardiovascular and Disease. London. HMSO.

De Smet, S., K. Raes and D. Demeyer, 2004. Meat fatty acid composition as affected by fatness and genetic factors: a review. *Anim. Res*., **53**: 81-98

Dikeman, M. E., 2007. Effects of metabolic modifiers on carcass traits and meat quality, Review Article. *Meat Science*, **77**: 121-135

Duran-Montge, P., C. A. Realini, A. C. Barroeta, R. Lizardo, and E. Esteve-Garsia, 2008. Tissue fatty acid composition of pigs fed different fat sources. *Animal,* **2** (12): 1753-1762.

Enser, M., R. I. Richardson, J. D. Wood, B. P. Gill and P. R. Sheard, 2000. Feeding linseed to increase the n-3 PUFA of pork: fatty acid composition of muscle, adipose tissue, liver and sosages. *Meat Science*, **55:** 201 – 212.

Guo, Q., T. Ritcher, J. R. Burgess, D. M. Webel, D. E. Orr, M. Blair, G. E. Pitzner, D. D. Hall, A. L. Grant and D. E. Gerrard, 2006. Effects of dietary vit. E and fat supplementation on pork quality. *J Anim. Sci*., **84**: 3089-3099.

Haak, L., K. Raes, S. De Smeet, E. Claeys, H. Paelink and S. De Smeet, 2006. Effect of dietary antioxidant and fatty acid supply on the oxidative stability of fresh and cooked pork. *Meat Science*, **74**: 476-486.

Haak, L., S. De Smeet, D. Fremaut, K. Van Walleghem and K. Raes, 2008. Fatty acid profile and oxidative stability of pork as influenced by duration and time of dietary linseed or fish oil supplementation. *J.Anim Sci*., **86**: 1418 – 1425.

Halenstvedt, E., N. P. Kjos, M. Overland and M. Thomassen, 2012. Changes in texture, color and fatty acid composition of male and female pig shoulder fat due to different dietary fat sources. *Meat Science*, **90**: 519 – 527.

Hertzman, C., L. Goransson and H. Ruderus, 1988.Influence of fish meal, rape-seed and rape-seed meal in feed on the fatty acid composition and storage stability of porcine body fat. *Meat Science*, **23**: 37-53.

Hoegberg, A., J. Pickova, K. Andersson and K. Lundstrom, 2003. Fatty acid composition and tocopherol content of muscle in pigs fed organic and conventional feed with different n-3/n-6 ratios respectively. *Food Chemistry*, **80**: 177 – 186.

Hoving-Bolink, A., H. Eikelenboom, G. van Diepen, J. T. M. Jongbloed, A. W. and J. H. Houben, 1998. Effect of dietary Vit. E Suplementation on pork quality. *Meat Science*, **49**: 205-212.

Juarez, M., M. E. R. Dugan, N. Alday, J. L. Aalhus, J. F. Patience, R. T. Zijlstra and A. D. Beaulieu, 2010. Feeding co-extruded flaxseed to pigs: Effects of duration and feeding level on growth performance and backfat fatty acid composition of grower-finisher pigs. *Meat Science*, **84**: 578-584

Kim, E. H., C. K. Han, K. S. Sung, C. S. Yoon, N. H. Lee, D. Y. Kim and C. J. Kim, 1994. Effect of Vit. E on lipid stability of pork meat. *Korean J. of Amin. Sci.,* **36**: 285-291.

Kouba, M., M. Enser, F. M. Whittington, G. R. Nutte and J. D. Wood, 2003. Effect of a high linolenic acid diet on lipogenic enzyme activities, fatty acid compoxition, and meat quality in the growing pig. *J. of Anim. Sci*., **81**: 1967-1979

Kouba, M. and J. Mourot, 2011. A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. *Biochemie*, **93**: 13 – 17.

Lauridsen, C. G., G. Andersen, M. Andersson, V. Danielsen, R. Engberg and K. Jakobsen, 1999. Effect of dietary fish oil supplied to pigs from weaning to 60 kg liveweight on performance, tissue fatty acid composition and palatability of pork when slaughtered on 100 kg live weight. *J. Anim. Feed Sci*., **8**: 441–456.

Leskanich, C. O., K. R. Mathews, C. C. Warkup, R. C. Noble and M. Hazledine, 1997. The effect of dietary oil containing n-3 fatty acids on the fatty acids, physicochemical and organoleptic characteristics of pig meat and fat. *Journal of Animal Science*, **75:** 673–683.

Liener, I. E., 1980. Toxic Constituents of Plant Feedstuffs. 2nd edn. *Academic Press,* New York, 502 pp.

Lu, P., L. I. Zhang, J. D. Yin, A. K. Everts and D. F. Li, 2008. Effect of Soybean oil and linsead oil on fatty acid compositions of muscle lipidsand cooked pork flavour. *Meat Science*, **80** (3): 910–918.

Monahan, F. J., D. J. Buckley, J. I. Gray, P. A. Morrissey, A. Asghar, T. J. Hahrahan and P. B. Lunch, 1990. Effect of dietary Vit. E on the stability of row and cooked pork. *Meat Sci*., **27**: 99-108.

Monahan, F. J., D. J. Buckley, P. A. Morrisey, P. B. Lunch and J. I. Gray, 1992. Influence of dietary fat and α - tokopherol supplementation on lipid oxidation in pork. *Meat Science*, **31**: 229-241.

Narayan, B., K. Miyashita and M. Hosokawa, 2006. Physiological effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) – A review. *Food Rev. Int*. **22**: 291 – 307.

Niedzwiedz – Siegien, I., 1998. Cianogenic glukosides in Linum Usitatissimum. *Phytochemistry*, **49** (1): 59 – 63.

Nuijten, W. G. M., P. C. H. Morel, J. Leong, R. W. Purchas and B. H. P. Wilkinson, 2011. Diet manipulation to produce pork with a greather concentration of long chain n-3 fatty acids. In: P. C. H. Morrel (Editor), Advancing Pork Production Seminar Palmerston North. (Proceedings of the Massey University 7th June vol. 2), pp. 163 – 174.

Niculita, P., M. E. Popa, M. Ghidurus and M. Turtoi, 2007. Effect of vit. E in swine diet on animal growth perfprmance and meat quality parameters. *Polish Journal of Food and Nutrition Sciences*, **57**: 125 – 130.

Ohkawa, N., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem*., **95**: 351 – 363.

Overland, M., O. Taugbol, A. Haug and E. Sundstol, 1996. Effect of fish oil on growth performance, carcass characteristics, sensory parameters and fatty acid composition in pigs. *Acta Agric. Scand. Sect. A, Anim. Sci*., **46**: 11 – 17.

Pikul, J., D. E. Leszczynski and F. A. Kummerow, 1893. Elimination of sample autoxidation by butylated hydroxytoluene additions before thiobarbituric acid assay for malonaldehyde in fat from chicken meat. *Y. Agric. Food Chem*., **31**: 1338 - 1344.

Raes, K., S. De Smet, and D. Demayer, 2004. Effect of dietary acid on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review, *Animal Feed Science and Technology*, **113**: 199-221

Rey, A. I., C. J. Lopez-Bote, J. P. Kerry, P. B. Lynch, D. J., Buckley and P. A. Morrissey, 2004. Modification of lipid composition and oxidation in porcine muscle microsomes as affected by dietary supplementation of n-3 with either n-9 n-6 fatty acids and α-tocopheryl acetate. *Animal Feed Science and Technology,* **113** (1 – 4): 223–238.

Riley, P. A., M. Enser, G. R. Nute and D. Wood, 2000. Effects of dietary linseed on nutritional value and other quality aspects of muscle and adipose tissue. *Animal Sci.,* **71**: 483-500

Romans, J. R., R. C. Johnson, M. V. Duane, G.V. Libal and W. J. Costello, 1995. Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega – 3 fatty acid contents of pork: II. Duration of 15% dietary flaxseed. *J. Anim. Sci.,* **73**: 1987-1999.

Santos, C., L. Hoz, M. I. Kambero, M. C. Gabeza and J. A. Ordonez, 2008. Enrichment of dry cured-ham with α-linolenic acid and α-tocopherol by the use of linseed oil and α-tocopheryl acetate in pig diets. *Meat Sci*., **80**: 668-674.

Shaw, D. T., D. W. Rozeboom, G. M. Hill, A. M. Boren and J. E. Link. 2002. Impact of vitamin and mineral supplement withdrawal and wheat middling inclusion on finishing pig growth performance, fecal mineral concentration, carcass characteristics, and the nutrient content and oxidative stability of pork. *J. Anim. Sci*., **80**: 2920-2930.

Specht – Overholt, S. M., J. R. Romans, M. J. Marchello, R. S. Izard, M. G. Grews, D. M Simon, W. J. Costello and P. D. Evenson, 1997. Fatty acid of commercially manufactured omega-3 enriched pork products, Haddock, and Mackerel, 1997. *Anim. Sci.,* **75**: 2235-2343.

Sprecher, H., D. L. Luthria, B. S. Mohammed and S. P. Baykousheva, 1995. Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. *J. Lipid Research*, **36**: 2471-2477.

Stat. Soft. Inc., 1994. Statistica for Windows, General Covention and Statistics. I. Stat. Soft. Inc., Tusla, USA.

Tikk, K., M. Tikk, M. D. Aaslyng, A. H. Karlsson, G. Lindahl and H. J. Andersen, 2007. Significance of fat supplemented diets on pork quality – Connections between specific fatty acids and sensory attributes of pork. *Meat Science,* **77**: 275-286.

Trefan, L., L. Bünger, J. Bloom-Hansen, J.A. Rooke, B. Salmi, C. Larzul, C. Terlouw and A. Doeschl-Wilson, 2011. Meta-analysis of the effects of dietary vitamin E supplementation on α-tocopherol concentration and lipid oxidation in pork. *Meat Science*, **87** (4): 305-31.

Van Oeckel, M. J., M. Casteels, P. Dirinck, N. Warnants and Ch.V. Boucque, 1995. Different aspects affecting the sensoty quality of pork. *Med. Fac. Landbouww. Univ. Gent*, 60/4a, pp. 1727 – 1736.

Van Oeckel, M. J., M. Casteels, N. Warnants, L. Wan Damme and Ch. V. Boucque, 1996. Omega – 3 Fatty Acids in Pig Nutrition: Implications for the Intrinsic Sensory Quality of the Meat. *Meat Science*, **44** (1-2): 55-63.

Warnants, N., M. J. V. Oeckel and C. V. Boucque, 1999. Incorporation of Dietary Polyunsaturated fatty Acid into Pork Fatty Tissues. **77**: 2478-2490

Waylan, A. T., P. R. O'Quinn, J. A. Unruh, J. L. Nelssen, R. D. Goodband, J. C. Woodworth, M. D. Tokach, and S. I. Koo. 2002. Effect of modified tall oil and vitamin E on growth performance, carcass characteristics, and meat qualityof growing finishing pigs. *Journal of Animal Science*, **80**: 1575-1585.

Wood, J. D., R. I. Richardson, G. R. Nute, A. V. Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard and M. Enser, 2003. Effect of fatty acids on meat quality: a review. *Meat Science*, **66**: 21–32.

Received December, 12, 2012; accepted for printing June, 2, 2013.