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The effect of grain polyphenols and the addition of vitamin E to diets enriched with α-linolenic acid on the antioxidant status of pigs*

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ABSTRACT

The aim of this study was to determine the antioxidant status and blood lipid profile of pigs fed diets enriched with α-linolenic acid (ALA) and containing two sets of antioxidants: phenolic compounds of diet components (barley, triticale, naked oat and buckwheat by-products) only, or phenolic compounds and vitamin E supplementation. A three-factorial experiment was performed on growing-finishing barrows (6-7 pigs per group) fed individually diets supplemented with 3% linseed oil, formulated as isoenergetic, isofibre and isofat. The influence of the type of diet (barley-triticale-based control diets vs 40%-oat-based diets), the share of buckwheat by-products (0 vs 10/12% of grower/finisher diets), and vitamin E supplementation (0 vs 100 IU·kg^{-1}) was evaluated. The analysed feed materials could be classified according to their total phenolic compound (TPC) content and *in vitro* antioxidant capacity (TAC) in the following decreasing order: buckwheat hulls and bran >> barley >> naked oat ≈ triticale. Diets with higher levels of TPC and TAC, i.e. barley-triticale-based control diets and diets containing buckwheat by-products, increased erythrocyte superoxide dismutase and glutathione peroxidase activity, similarly as 100 IU·kg^{-1} vitamin E supplementation, thus suggesting an improvement in the antioxidant status of pigs. Only vitamin E supplementation significantly (over four-fold) increased vitamin E concentrations in the blood serum and *M. longissimus dorsi* (LD) of pigs, and decreased the rate of lipid oxidation in LD. The obtained results suggest that the phenolics present in cereal grains and in buckwheat, occurring as

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natural antioxidants in ALA-rich and vitamin E-deficient diets, are not sufficient to maintain the oxidative stability of pork. Diets containing 40% oat significantly improved the HDL/TCH and HDL/LDL ratios in serum.

KEY WORDS: grains, buckwheat, phenolics, vitamin E, α-linolenic acid, antioxidant status, serum lipids, meat, pigs

INTRODUCTION

In the typical Western diet, the intake of n-3 PUFAs is lower relative to n-6 PUFAs. The dietary n-6/n-3 PUFA ratio ranges between 15:1 and 17:1 in Western Europe and in the United States, whereas nutritional recommendations suggest a value 4:1 or less (Simopoulos, 2002). One of the solutions could be to enrich foods of animal origin in deficient n-3 fatty acids, mainly α-linolenic acid (C18:3n-3, ALA). The ALA content of pork may be increased by offering diets supplemented with linseed or linseed oil to growing-finishing pigs. A high PUFA diet may, however, enhance oxidative stress in the body because PUFA are highly susceptible to peroxidation. Therefore, an increase in n-3 PUFA intake should be accompanied by diet supplementation with antioxidants. Synthetic α-tocopherol (vitamin E) is the most commonly used antioxidant in animal nutrition. Natural antioxidants, which may augment the effects of vitamin E or substitute for it, are readily accepted by both consumers and health authorities (Pokorný, 2007).

Grains possess significant antioxidant capacity derived from hydrolysable polyphenols associated with dietary fibre, studied mostly in the aspect of human nutrition (Vitaglione et al., 2008). Among cereals, oat grain has been extensively investigated and has been shown to contain many compounds that exhibit antioxidant properties, such as tocopherols, tocotrienols, flavonoids, phenolic acids, avenantramides and sterols (Peterson, 2001). A major part of the antioxidant capacity (>95%) of oat is derived from phenolic compounds in the aleurone, and only <5% from tocols (Handelman et al., 1999). In a study on broiler chickens, partial replacement of maize with oats increased the oxidative stability of fat in meat (Lopez-Bote et al., 1998). Our previous experiment also showed an increase in the oxidative stability of fresh loin from pigs fed a diet containing 45% naked oat instead of triticale, whereas no such effect was noted in pigs fed husked oat (Flis et al., 2007).

The pseudocereal buckwheat has received renewed interest due to, among others, its content of phenolic compounds (flavanols, flavonoids) with antiperoxidative potential (Quettier-Deleu et al., 2000). During the production of roasted groats (known in Poland as kasha), large quantities of buckwheat by-products, such as hulls and bran, are formed. Compared with groats, hulls contain more flavonoids (rutin, quercetin) than flavanols (Quettier-Deleu et al., 2000). Studies on human volunteers

(Bojňanská et al., 2009) have shown that buckwheat phenolics increased the body's antioxidant status. The question is, therefore, whether phenolic compounds, not classified as vitamins, can be effective as antioxidants in pig diets rich in n-3 PUFA.

The objective of this experiment was to determine the antioxidant status and blood lipid profile of pigs fed diets enriched with α-linolenic acid and containing two sets of antioxidants, i.e. only phenolic compounds of diet components (barley, triticale, naked oat and buckwheat by-products) or phenolic compounds and vitamin E supplementation.

MATERIAL AND METHODS

Feed materials

Naked oat, barley and triticale grain were purchased from a local farmer. Buckwheat by-products, i.e. hulls (18 to 20% of total kernel weight) and bran (15-17% of total kernel weight), were purchased from a local producer of roasted buckwheat groats (Melvit S.A. Kaszarnia Szczytno, Poland). Buckwheat bran consists of crushed groats, flour, and small fragments of hulls. Unrefined linseed oil, rapeseed oil (supplied by the oil producer, Plant of Oil Pressing, Grodzisk Wlkp., Poland), and soyabean oil (LNB, Poland) were used. Arbocel RC lignocellulose concentrate was obtained from Rettenmaier and Söhne (Germany), and Microvit™ E Promix 50 (Commenty, France) from Adisseo (Poland). The other ingredients of the diets were purchased from a local feed manufacturer (LNB, Poland).

Animals, diets and sampling

Fifty-two crossbred barrows [(Polish Large White × Polish Landrace) × Duroc] were purchased from a commercial piggery, and were housed individually in cages equipped with feeders and nipple drinkers. During the adaptation period (7 days) pigs were fed the same diet, and then they were allocated to eight groups (6-7 animals per group) based on average body weight (BW). The experiment was conducted in a 2 × 2 × 2 factorial design on pigs with an initial body weight of 45.9±3.2 kg, fed grower diets for 37 days (to approximately 77 kg BW), followed by finisher diets (to 108±2.7 kg BW).

The influence of two types of diets (C, control diets and O, oat-based diets), the share of buckwheat by-products (0 or 100/120 $g \cdot kg^{-1}$ of grower/finisher diets), and vitamin E supplementation (0 or 100 $IU \cdot kg^{-1}$) was evaluated. Apart from soyabean meal, C diets were composed of barley and triticale, which are

typically fed to pigs in Poland. O diets contained 400 g·kg^{-1} naked oat, which partially replaced the above two cereal species (Table 1). The diets were formulated as rich in ALA, therefore 30 g·kg^{-1} linseed oil and 10 g·kg^{-1} rapeseed

Table 1. Ingredients content in grower and finisher diets used in growth experiment on pigs, g·kg^{-1}

Ingredients	Grower, from 46 to 77 kg BW				Finisher, 77 to 108 kg BW			
	C[1]	CB[1]	O[1]	OB[1]	C[1]	CB[1]	O[1]	OB[1]
	CE[5]	CBE[5]	OE[5]	OBE[5]	CE[5]	CBE[5]	OE[5]	OBE[5]
Barley	311.5	291.7	147.0	130.9	363.4	323.5	196.6	166.6
Triticale	320	290	150	130	350	320	190	160
Naked oat	-	-	400	400	-	-	400	400
Buckwheat bran + hulls (1:1)	-	100	-	100	-	120	-	120
Soyabean meal	227	225	175	165	163	160	102	97
Linseed oil	30	30	30	30	30	30	30	30
Rapeseed oil	21	21	10	10	11	11	-	-
Soyabean oil	10	10	-	-	11	11	-	-
Arbocel [2]	48	-	54	-	47	-	55	-
Mineral feeds	17	17	18	18	9	9	10	10
Mineral and vitamin premix [3]	10	10	10	10	10	10	10	10
L-Lysine, 78%	1.1	0.9	1.7	1.8	1.3	1.25	2.2	2.2
DL-Methionine, 98%	0.4	0.4	0.3	0.3	0.3	0.25	0.2	0.2
Acidifier [4]	4	4	4	4	4	4	4	4
E-Promix 50 [5], mg·kg^{-1}	200	200	200	200	200	200	200	200

[1] C - control diet; CB - control diet with buckwheat by-products; O - oat diet; OB - oat diet with buckwheat by-products; [2] Arbocel RC as a source of fibre; [3] vitamin-trace mineral premix on limestone carrier, without vitamin E, supplied per kg diet: IU: vit. A 10 000, vit. D_3 2000; mg: vit. K_3 1.50, folic acid 2, panthothenic acid 10, niacinamid 20, thiamine 1.50, riboflavine 4.0, pyridoxine 3.0, cholinchloride 300, Fe 100, Mn 40, Cu 20, Zn 100, I 1.2, Co 0.6, Se 0.12; µg: vit. B_{12} 25, biotine 100; [4] acidifier containing the mixture of acids (formic, phosphoric, acetic, lactic, citric, propionic) and their salts; [5] diet denoted CE, CBE, OE and OBE were supplemented with 100 IU·kg^{-1} vitamin E by addition of Microvit E-Promix 50, consisting all-*rac*-α-tocopheryl acetate (vitamin E content 500 IU·g^{-1}) on expanded silica carrier

oil were introduced into the grower diets, and 30 g·kg^{-1} of linseed oil was incorporated into the finisher diets. The diets were also formulated as isoenergetic and had the same crude fat content (rapeseed oil and soyabean oil were added to C diets to achieve the fat level of O diets). Since fibre may reduce the bioavailability of antioxidants in the gut, Arbocel was introduced to obtain the crude fibre level of diets with buckwheat by-products. The vitamin-trace mineral premix used in all diets was not supplemented with vitamin E. Diets C, CB, O, and OB contained only natural sources of vitamin E from dietary feed materials. Vitamin E-enriched diets (CE, CBE, OE, OBE) were formulated by adding 100 mg·kg^{-1} all-*rac*-α-tocopheryl acetate (Microvit^{TM} E-Promix 50) which is equivalent to 100 IU·kg^{-1} vitamin E. The oil used to prepare finisher diets was stored in plastic containers

in a refrigerated cabinet. The diets were mixed in a small (500 kg) feed mixer. Laboratory samples were collected and analysed from every batch of the diets. The diets were offered wet (mixed with water at a ratio of 1:1), in a restricted amount (from 1.8 kg in the 1st week to 3.0 kg/pig/day in the last week of the experiment).

After 61 days of experimental feeding, blood samples were collected on two consecutive days from pigs deprived of feed for 16 h, in two replicates (from half of the animals each day), by puncture of the vena cava. One set of samples, transferred into heparinized tubes stored on ice, was used for the determination of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in erythrocytes on the same day. In the second set of samples, the serum was separated by centrifugation at 1 500 *g* for 15 min at 4°C and stored at -40°C until analysis of total antioxidant status (TAS), α-tocopherol, total cholesterol, triglycerides, and HDL-cholesterol.

As pigs achieved slaughter weight they were transported to a slaughterhouse (in two groups, at one-week intervals) and after around three h they were slaughtered following carbon dioxide stunning. Twenty-four h after slaughter, samples of chilled dissected *M. longissimus dorsi* (LD) were collected from the area above the third and fourth lumbar vertebra. After transportation in a cooler to the laboratory, every LD sample was divided into two parts. One set of samples was placed into impermeable plastic bags and frozen at -18°C for four months for the evaluation of thiobarbituric acid reactive substances (TBARS). The fresh set of samples was used for the analysis of α-tocopherol, TBARS, and fatty acid content.

The experimental procedures used in this study were approved by the Animal Care and Use Committee at the University of Warmia and Mazury.

Analytical methods

Nutrient contents of diets. The concentrates (diets) were analysed for nutrient content according to AOAC (2003) procedures.

Fatty acid composition. The fatty acid composition of diets and meat samples was analysed after methylation of extracted lipids (Peisker, 1964), in a gas chromatograph (AT 6890 N, Palo Alto, CA USA), on a silica gel capillary column (30 m × i.d. 0.32 mm; 0.25 μm film thickness). The injector, detector and column temperatures were maintained at 225, 250 and 180°C, respectively. The liquid phase was Supelcowax10. Peaks were identified based on their retention times, corresponding to the reference material CRM 163 beef-pig fat blend (Promochem, Warszawa, Poland). The results were expressed as the percentage of the total fatty acid composition of diets and LD.

α-Tocopherol in feed materials was determined by HPLC (Shimadzu, Japan) following the procedure of PN-EN-ISO (2002). Serum α-tocopherol levels

were measured by the method of McMurray and Blanchflower (1979), and the α-tocopherol content of meat samples was determined by Rettenmaier and Schüep (1992). The peaks were quantified by calibration with the all-*rac*-α-tocopherol standard (Sigma, Switzerland).

Total phenolic compounds (TPC) from 80% methanol extracts were determined by the method of Shahidi and Naczk (1995), with the Folin-Ciocalteu reagent, at 725 nm (UV-160 IPC spectrophotometer, Shimadzu). The results were expressed as ferulic acid equivalents.

The antioxidant capacity (TAC) of feed materials was determined *in vitro* based on the reduction of the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) by antioxidants present in feed, using a method described by Brand-Williams et al. (1995). DPPH scavenging activity was expressed in terms of Trolox equivalent capacity, using the Trolox standard curve, based on the percentage inhibition of absorbance at 734 nm of standards and samples (UV-160 IPC spectrophotometer, Shimadzu).

In vivo antioxioxidant properties. Superoxide dismutase (EC 1.1.5.1; SOD) and glutathione peroxidase (EC 1.11.1.9; GSH-Px) activities in erythrocyte lysates were assayed using kits from Randox Laboratories Ltd. (Crumlin, UK). SOD activity was measured by the method of Woolliams et al. (1983), and GSH-Px activity by the method of Paglia and Valentine (1967). The serum total antioxidant status (TAS) was measured using a Randox kit, according to the procedures of Miller et al. (1993). TBARS values were evaluated to determine the effect of experimental diets on oxidative stability in meat samples on day 1 and after four-month frozen storage. TBARS values were determined as described by Sørensen and Jorgensen (1996).

Serum lipids. The serum concentrations of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL) were determined enzymatically using commercially available kits (Pointe Scientific, Poland) and a spectrophotometer (Specol 11, Carl Zeiss Jena, Germany). Low-density lipoprotein cholesterol (LDL) was calculated according to the formula LDL= TCH−HDL-TG/2.2 (Friedwald et al., 1972). The serum percentage HDL/TCH and HDL/LDL ratios were also calculated.

α-T, TPC and TAC values were determined in three replications in laboratory samples of feed materials. The FA content of diets and the LD muscle, α-T, TAS, SOD, GSH-PX, TCH, TG, HDL levels in the blood, and TBARS values in the LD muscle were determined in two replications.

Statistical analysis

A three-way non-orthogonal (6 and 7 pigs, in the groups with and without

vitamin E supplementation, respectively) analysis of variance was performed using StatSoft STATISTICA, version 7.1. Significant differences between the experimental factors were determined by Duncan's multiple-range test. All tables contain means and the standard error of means (SEM).

RESULTS

The analysed feed materials differed with respect to α-T and TPC content and TAC values (Table 2), which affected the concentrations of the above compounds and TAC values in experimental diets (Table 3).

Table 2. α-Tocopherol (α-T), total phenolic compounds (TPC) and total antioxidant capacity (TAC) of feed materials

Feed	α-T mg·kg^{-1}	TPC g·kg^{-1}	TAC mmol·kg^{-1}
Barley	3.40	2.05	5.67
Triticale	3.17	0.90	2.23
Naked oat	6.46	1.14	2.51
Soyabean meal	4.47	3.92	4.04
Buckwheat bran	3.16	5.47	8.09
Buckwheat hulls	1.82	5.92	8.10
Linseed oil	172.4		
Rapeseed oil	69.1		
Soyabean oil	113.7		
Microvit E-Promix 50	528 500		

Pigs from all groups showed high performance values (data not shown). Due to restricted feeding, average daily feed intake was comparable in all groups (2.52-2.54 kg/day/pig). Daily gains (873±63 g) and the feed-to-gain ratio (2.91±0.22 kg/kg) did not differ significantly among the dietary treatments during the growing-finishing period.

Pigs fed control diets, which contained higher amounts of barley and triticale, had a significantly better antioxidant status, measured by erythrocyte SOD activity ($P<0.05$), GSH-Px activity ($P<0.001$), and by TAS ($P<0.05$), compared with pigs fed diets with 40% naked oat (Table 4). However, pigs given oat-based diets had higher serum concentration of α-T ($P<0.01$), compared with pigs fed control diets. The introduction of buckwheat by-products into the diets increased SOD activity ($P<0.01$), and tended to increase GSH-Px activity ($P=0.076$) in pigs' blood. Diets supplemented with vitamin E increased SOD activity ($P<0.001$) and α-T concentration ($P<0.001$) in the blood.

Table 3. Nutritional value, main fatty acids and antioxidants content in the grower and finisher diets

Item	Grower diet [1, 2]				Finisher diet [1, 2]			
	C, CE	CB, CBE	O, OE	OB, OBE	C, CE	CB, CBE	O, OE	OB, OBE
Nutritional value, % as fed								
crude protein	18.00	18.06	18.08	18.12	15.39	15.64	15.40	15.91
ether extract	7.15	7.21	7.53	7.47	5.98	6.15	6.34	6.57
crude fibre	6.13	6.57	5.91	6.47	6.36	6.92	6.49	6.43
gross energy, MJ/kg	17.41	17.45	17.55	17.56	17.38	17.44	17.57	17.83
ME, MJ/kg	13.35	13.33	13.25	13.21	13.24	13.07	13.08	13.01
Fatty acid, % of total FA								
∑ SFA	12.3	12.4	14.5	14.4	13.9	14.3	15.5	15.9
∑ MUFA	31.8	32.3	33.8	33.6	27.0	27.8	29.2	29.9
∑ PUFA	56.0	55.4	51.7	52.0	59.1	57.9	55.3	54.2
ALA	29.5	27.8	25.5	25.9	30.8	29.7	28.0	26.6
Antioxidant								
α-tocopherol [3], mg·kg^{-1}	10.9	10.9	10.2	10.3	10.3	10.3	9.5	9.6
TPC, g·kg^{-1}	1.82	2.31	1.54	2.01	1.70	2.26	1.39	1.96
TAC [4], mmol·kg^{-1}	3.40	4.02	2.87	3.50	3.50	4.17	2.95	3.66

[1, 2] for explanation see Table 1; [3] α-tocopherol content in the diet calculated from amount analysed in feed materials, without consideration of amount incorporated by Microvit E Promix 50; [4] as a Trolox equivalent

Table 4. Effect of dietary grain and buckwheat polyphenols, and vitamin E supplementation on blood antioxidant status of pigs

Item	SOD units/ml	GSH-Px units/ml	TAS mmol/l	α-T μg/ml
Diet (D)				
C-control	145.1	26.00	1.12	0.72
O-oat	133.1	23.33	1.03	0.88
Buckwheat by-products (B)				
0	131.2	24.06	1.09	0.78
10/12% [1]	147.0	25.38	1.07	0.82
Vitamin E supplement (E)				
0	129.6	24.25	1.06	0.33
100 IU·kg^{-1}	150.5	25.27	1.09	1.37
SEM	3.12	0.463	0.018	0.085
P value				
D	<0.05	<0.001	<0.05	<0.01
B	<0.01	0.076	NS	NS
E	<0.001	NS	NS	<0.001
Significancy of interaction		D × B* D × E*		D × E*

[1] in the grower/finisher diet; NS-not significant;* P≤0.05

LD lipids from all groups showed high accumulation of PUFAs in total fatty acids (≈11%), including ALA (≈4%), and the recommended ratio of C18:2/C18:3 (below 2) (Table 5).

Table 5. Main fatty acids of the *M. longissimus dorsi*, % of total FA

Item	C18:0	C18:1	C18:2	C18:3	∑SFA	∑ MUFA	∑ PUFA	C18:2/ C18:3
Diet (D)								
C-control	12.32	43.77	7.03	4.26	39.98	48.24	11.36	1.67
O-oat	13.17	43.38	6.95	3.51	41.31	47.71	10.97	1.97
Buckwheat by-products (B)								
0	12.47	43.87	6.91	3.83	40.53	48.22	10.83	1.82
10/12% [1]	12.75	43.29	7.06	3.91	40.79	47.73	11.48	1.81
Vitamin E supplement (E)								
0	12.91	43.31	7.07	3.93	40.83	47.65	11.52	1.81
100 IU·kg^{-1}	12.58	43.88	6.89	3.80	40.46	48.34	10.75	1.84
SEM	0.173	0.317	0.241	0.128	0.344	0.350	0.438	0.039
P value								
D	0.019	NS	NS	0.006	NS	NS	NS	<0.01
B	NS	NS	NS	NS	NS	NS	NS	NS
E	NS	NS	NS	NS	NS	NS	NS	NS

[1] in the grower/finisher diet

The effect of experimental treatments on the antioxidant status of pigs was also verified based on α-T concentration and the formation of TBARS in meat (Table 6). α-T concentration in the LD samples from pigs fed diets containing high PUFA levels and only natural sources of vitamin E was very low, and they increased ($P<0.001$) in pigs fed vitamin E-supplemented diets. The use of oat grain and buckwheat by-products in the diets did not increase the α-T content of pork. After slaughter, TBARS concentration was lower in the meat from pigs fed control diets, compared with those fed oat-based diets ($P<0.05$). However, during four-month storage, TBARS values were similar in the meat from pigs fed control and oat-based diets. Dietary vitamin E supplementation reduced the rate of lipid oxidation in fresh ($P=0.067$) and stored ($P<0.05$) meat.

After two months of experimental treatment, pigs fed O diets had significantly higher HDL/TCH ($P<0.01$) and HDL/LDL ($P=0.011$) ratios, in comparison with pigs that received control diets (Table 7).

Table 6. Effect of dietary grain and buckwheat polyphenols or vitamin E supplementation on α-tocopherol concentration and lipid oxidation in *M. longissimus dorsi*

Item	α-tocopherol mg·kg^{-1}	TBARS, mg·kg^{-1}	
		day 1	after 4-monthly storing
Diet (D)			
C-control	1.14	0.596	1.098
O-oat	1.12	0.687	1.075
Buckwheat by-product (B)			
0	1.13	0.656	1.091
10/12% [1]	1.13	0.627	1.082
Vitamin E supplement (E)			
0	0.47	0.676	1.228
100 IU·kg^{-1}	1.92	0,599	0.917
SEM	0.122	0.021	0.060
P value			
D	NS	<0.05	NS
B	NS	NS	NS
E	<0.001	0.067	<0.05
Significancy of interaction	D×B**		
	D×B×E*		

[1] in the grower/finisher diet; *P≤0.05; **P≤0.01

Table 7. Effect of dietary grain, buckwheat by-products polyphenols, and vitamin E supplementation on serum lipids profile in pigs

Item	TCH mmol/l	HDL mmol/l	TG mmol/l	LDL mmol/l	HDL/TCH %	HDL/LDL %
Diet (D)						
C-control	2.93	1.16	0.29	1.64	39.6	72.9
O-oat	2.84	1.23	0.33	1.46	43.7	89.7
Buckwheat by-product (B)						
0	2.86	1.19	0.35	1.51	42.1	85.1
10/12% [1]	2.91	1.19	0.28	1.59	41.2	77.5
Vitamin E supplement (E)						
0	2.93	1,19	0.27	1.62	40.9	77.0
100 IU·kg^{-1}	2.83	1.20	0.37	1.47	42.5	86.5
SEM	0.067	0.025	0.026	0.058	0.73	3.34
P value						
D	NS	NS	NS	0.145	<0.01	0.011
B	NS	NS	NS	NS	NS	NS
E	NS	NS	0.051	NS	NS	0.130

[1] in the grower/finisher diet; NS - not significant

DISCUSSION

Due to differences in TPC and TAC levels in feed materials, the control diets contained more TPC and had higher TAC (by 15% on average) than oat-based diets (Table 3). Greater increases in dietary TPC (by 33% on average) and in TAC (by 21%) were noted as buckwheat by-products were introduced into the diets. Differences in the levels of TPC and TAC in diets with high concentration of PUFA (including ALA), and deficient in α-tocopherol, were expected to influence the body's antioxidant status.

Phenolic compounds posses potent antioxidant activity, and may directly remove peroxidation inhibitors by quenching chain-initiating catalysis, or by the chain-breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the system, e.g., a lipid radical (Vaya and Aviram, 2001). The results of this study show that higher levels of phenolic compounds in control diets (with an increased barley content) and in diets with buckwheat by-products, increased, similarly as vitamin E supplementation, the activities of antioxidant enzymes in erythrocytes (SOD, GSH-Px). This suggests that due to the increased content of polyphenols or vitamin E in the diets, endogenous antioxidants were less involved in the reduction of reactive oxygen species and lipid peroxide radicals, thus improving the blood antioxidant status of pigs. An improvement in the redox state of animals fed diets with polyphenol-rich cereals or supplemented with vitamin E, by increasing blood antioxidant enzyme activity, was reported by Álvarez et al. (2006).

In the current study, pigs fed diets without vitamin E supplementation, containing only approximately 10 $mg \cdot kg^{-1}$ natural α-T, had very low α-T concentration in serum (0.33 $\mu g \cdot ml^{-1}$) and in the LD meat (0.47 $mg \cdot kg^{-1}$). This could be due to the presence of high PUFA and ALA levels in the diets, exceeding 50 and 26% total fatty acids, respectively. In our study, pigs received experimental diets over a long period of time, i.e. for 61 days until blood sample collection, and for on average 71 days until slaughter. In experiments of Asghar et al. (1991a,b), α-tocopherol concentrations in the plasma and LD muscle of pigs fed for 60 days a diet with 6% soyabean oil and without addition of vitamin E were also low, at 0.43 $\mu g \cdot ml^{-1}$ and 0.5 $mg \cdot kg^{-1}$, respectively. We expected that the dietary supplementation of the antioxidant compounds contained in oat and buckwheat by-products to increase vitamin E concentrations in the blood and in muscles. However, neither oat-based diets nor diets with buckwheat by-products increased α-T levels in the LD muscle, and only oat diets increased (by 22%) serum α-T, compared with control diets. Similarly as in our study, also Augustin et al. (2008) reported that dietary supplementation of growing pigs with green tea polyphenols did not affect serum, liver and muscle vitamin E (α- and γ-tocopherol) concentrations in pigs fed diets

containing 17 IU·kg^{-1} vitamin E over a period of five weeks.

The application of 100 IU·kg^{-1} vitamin E considerably (over four-fold) increased the concentrations of α-T in the blood serum and in the LD muscle of pigs. Owing to α-T accumulation, the antioxidant status of the LD muscle (measured by TBARS formation) in pigs fed vitamin E-supplemented diets tended to be better (P=0.067) on the first day after slaughter, and it was significantly improved after four months frozen storage. Except for the higher oxidative stability of fresh meat (day 1) from pigs fed control diets, dietary TPC without vitamin E supplementation did not increase the muscle antioxidant status. α-Tocopherol is a major antioxidant localized in muscle lipids whose level is an important contributor to the resistance of lipids to oxidation. Higher dietary intake of α-tocopherol is accompanied by its higher accumulation in muscles. In our study increased TPC intake, in particular from a diet containing buckwheat by-products, did not decrease lipid oxidation in the LD muscle, which may suggest that dietary phenolics and their metabolites are not accumulated in muscles, or are accumulated in trace amounts only. This conclusion is consistent with the findings of Gonzalez and Tejeda (2007). The cited authors found that supplementing pig diets containing 20 mg·kg^{-1} α-tocopheryl acetate with a commercial flavonoid extract or with phenolic-enriched extract did not increase α-tocopherol concentrations in the LD muscle. None of the investigated extracts increased the concentrations of total phenols in the muscle, and therefore meat from pigs fed flavonoids and phenolic-enriched diets had similar TBARS values as meat from pigs fed a control diet, but significantly higher than meat from pigs fed α-tocopherol-enriched diets. Very low concentration of quercetin in the LD muscle of pigs was also reported by Bieger et al. (2008) who did not observe any direct antioxidant effect of flavonols in meat, relevant for post-mortem storage and stability. The largest amounts of quercetin were noted in the organs involved in metabolism, and quercetin concentration was higher in the blood plasma than in the LD muscle.

In our *in vitro* tests, naked oat exhibited lower antioxidant capacity than barley. The results of *in vivo* studies (blood SOD and GSH-Px activity, TAS, TBARS in meat) also suggest that the antioxidant potential of diets with 40% oat was lower than that of control diets with a higher barley content. The obtained results could have also been affected by slight differences in the linolenic acid content of diets. Our results correspond with the findings of Homb and Matre (1986) who found that oat diets decreased the oxidative stability of pig fatty tissue, measured by MDA formation, compared with barley diets.

In the current experiment, diets containing 40% oat had a more beneficial influence on the serum lipid profile than control diets without oat. Oat-based diets significantly increased the HDL/TCH ratio (from 39.6 to 43.7%) and the HDL/LDL ratio (from 72.9 to 89.7%), thus confirming the antiatherogenic

effects of oat. Since control diets and diets with a 40% share of naked oat contained similar levels of crude fibre, the obtained results could have been affected by a different content of soluble dietary fiber, including soluble β-glucan. According to Delaney et al. (2003), not only oat β-glucan, but also barley β-glucan is antiatherogenic. However, assuming a β-glucan content of 3.6% in barley and oat grains, and of 1% in triticale grains (Bach Knudsen, 1997), the administered oat diets had an approximately 45% higher β-glucan content than control diets (21.7 vs 14.8 $g \cdot kg^{-1}$ on average). The different solubility and molecular weight of oat and barley β-glucan could have also influenced the obtained results.

Owing to their high TPC content and high antioxidant capacity, buckwheat by-products (hulls, bran) may be valuable components of pig diets. Many feed materials with a high fibre content are recommended not only for gestating sows but also for piglets. Buckwheat hulls and bran, obtained from the hydrothermal treatment of whole grain, may be a rich source of both insoluble fibre and antioxidant compounds.

CONCLUSIONS

The analysed diet components could be classified according to their total phenolic compound (TPC) content and *in vitro* antioxidant capacity (TAC) in the following decreasing order: buckwheat hulls and bran >> barley >> naked oat ≈ triticale. Diets with higher levels of TPC and TAC improve the blood redox state (increased superoxide dismutase and glutathione peroxidase activity) of pigs fed α-linolenic acid (ALA)-rich but vitamin E-deficient diets. Only 100 $IU \cdot kg^{-1}$ vitamin E supplementation increased α-tocoferol concentrations in the blood serum and *M. longissimus dorsi* of pigs, and it decreased the rate of lipid oxidation in pork during storage. The obtained results suggest that the phenolics present in cereal grains and in buckwheat are not sufficient to maintain the oxidative stability of pork from pigs fed ALA-enriched diets. Diets with a 40% oat content significantly increased serum HDL/TCH and HDL/LDL ratios. The obtained results indicate that phenolic compounds and antioxidant capacity may become new measures for the nutritional value of pig diets.

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