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EFFECT OF VEGETABLE OILS SUPPLEMENTATION IN PIG DIETS ON LIPID OXIDATION AND FORMATION OF OXIDIZED FORMS OF CHOLESTEROL IN MEAT

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The effects of giving pigs dietary vegetable oils on oxidative stability, cholesterol level and oxysterol formation in their meat were studied. A total of 40 Polish Landrace pigs were randomly allocated to 4 groups with 5 gilts and 5 barrows per group and fattened from 50 to 105 kg body weight. Fat supplements represented the experimental factor: palm oil, linseed oil, rapeseed oil, and sunflower oil given at 3% of ration dry matter. A significantly higher MUFA level was found in the *m. longissimus dorsi* of pigs receiving dietary palm oil compared to the linseed oil-fed pigs ($p<0.05$). There was a highly significant narrowing in the *n-6*/*n-3* PUFA ratio between the experimental groups ($p<0.01$). In addition, the level of DHA acid was significantly higher in gilts than in barrows ($p<0.01$). The use of dietary vegetable oils caused a significant decrease in the oxidative stability of meat, in particular after 180 days of frozen storage of meat ($p<0.01$). A highly significant interaction was found for TBARS between the fat supplement used and sex ($p=0.003$). There were highly significant differences in vitamin E content of meat between the group receiving palm oil and the linseed oil-fed group ($p<0.01$). The type of oil had no significant effect on the total cholesterol content of meat. It was found that oxidized forms of cholesterol formed during storage. There were highly significant differences in the level of 7-ketocholesterol between the groups receiving palm oil and sunflower oil and the groups fed linseed oil and rapeseed oil ($p<0.01$). A similar, highly significant correlation was found between the level of total oxysterols and total cholesterol, with additional differences between the groups receiving linseed oil and rapeseed oil ($p<0.01$). Highly significant interactions, ranging from $p=0.002$ to $p=0.08$, were found between the level of oxysterols, and the source of fat and sex.

INTRODUCTION

Products of animal origin are usually characterised by high fat content, in which saturated fatty acids (SFA) dominate. However, the composition of fatty acids in animal food, including pork, can be modified by altering the pool of fatty acids in feed [Warnants *et al.,* 1999; Pieszka *et al.,* 2004]. Several studies have confirmed the possibility of incorporating feed fatty acids into body tissues, which affects the quantitative and qualitative traits of meat [Wiseman *et al.,* 2000; Wood *et al.,* 2004]. One of the ways of enriching meat with *n-3* polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) is to use vegetable oils in pig diets as a source of these acids [López-Bote *et al.,* 2002]. Diets for pigs raised in Poland are most often based on cereals, mainly barley and triticale, which are characterised by a high level of linoleic acid (C18:2), which is an *n-6* acid. As a result, the level of *n-6* PUFA in meat increases as the level of *n-3* acids decreases, which is not favourable from the viewpoint of consumer health. Pork, which has relatively high levels of fat, cholesterol and iron, undergoes oxidation processes relatively easily [Igene & Pearson, 1979]. The use in pig diets of feeds rich in unsaturated fatty acids (UFA), including plant fats, is beneficial for consumer health as these acids improve the dietetic value of meat. However, excessive UFA levels in animal fat reduce the oxidative stability of lipids, which may negatively affect the sensory traits (flavour and aroma) and storage life of meat [Leksanich *et al.,* 1997; Mottram, 1998; Rey *et al.,* 2001a]. Such meat and meat products are characterised by shorter shelf life determined by a greater susceptibility of PUFA to the oxidation processes, even after the meat has been frozen [Buckley *et al.,* 1995; Sheard *et al.,* 2000; Pieszka *et al.,* 2006].

Cholesterol is oxidized in a similar way to UFA and phospholipids. The oxygenated derivatives of cholesterol (known as oxysterols) are mainly formed during the cooking, frying, grilling and storage of meat [Panianghwait *et al.,* 1995]. Cholesterol oxidation products (COP) are considered one of the causes of atherosclerotic changes in humans as they have a negative impact on the cardiovascular system [Kumar & Singhal, 1992] and damage the central nervous system, which is one of the causes of Alzheimer's and Parkinson's diseases [Halliwell, 1992].

Because of its relatively high level of iron and adequate level of cholesterol, meat is acknowledged as a potential source of oxysterols [Kumar & Singhal, 1992]. Smith [1996] postulated that cholesterol oxidation may take place in a similar way to the oxidation of fatty acids. The high level of PUFA in food phospholipids and the fact that they are unprotected from the attacks of oxidative compounds within cells and near cell membranes may trigger lipid oxidation at the intracellular membrane [Igene & Pearson, 1979]. Smith

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[1996] suggested that the hydroxyperoxidation of PUFA, that takes place during lipid oxidation, may be necessary for triggering the cholesterol oxidation process.

Furthermore, results of some studies [López-Bote *et al*., 2002; Daza *et al.,* 2005] suggest that different classes of fatty acids (SFA, UFA, MUFA, *n-6* PUFA, *n-3*PUFA) in pig diets have varying effects on tissue desaturase activity, as reflected in the level of particular fatty acids in lipids and the level of cholesterol.

The aim of the study was to determine the effect of giving pigs vegetable oils with different MUFA and PUFA levels on lipid oxidation, cholesterol level and formation of the oxidized forms of cholesterol in meat.

MATERIAL AND METHODS

A total of 40 Polish Landrace pigs were randomly allocated to 4 groups with 5 gilts and 5 barrows per group. Animals were fattened from 50 to 105 kg body weight using a feed mixture containing a 3% supplement of vegetable oils. Fat supplements represented the experimental factor: palm oil, linseed oil, rapeseed oil and sunflower oil given at 3% of ration dry matter. The complete diets contained wheat, barley, triticale, soybean meal, mineral additives, standard premix (Lutamix complete NP, BASF Kutno) and vegetable oils.

The basic and amino acid composition of the diets was determined using standard methods [AOAC, 1995]. The nutritive value of the diets and the composition of fatty acids in the oils used are presented in Table 1. Metabolizable energy was calculated based on the composition of the diet, accounting for tabular values for particular components [Polish Nutrient Requirements,1993].

Animals were kept in individual pens equipped with nipple drinkers and fed *ad libitum*. On the day before slaughter, animals were fasted with free access to water and transported to a slaughterhouse. Pigs were slaughtered in a standard slaughterhouse after electrical stunning. Following 24-h chilling of carcasses at 4°C, meat samples were taken from the *m. longissimus dorsi* near the 4th and 5th lumbar vertebrae, secured and stored at 19°C. Meat samples were analysed for crude fat using a Büchi 810 Soxhlet apparatus (Flawil, Switzerland) according to the method described by Budsławski & Drabent [1972].

Chemical reagents. The following solvents were used for the analyses: methanol, acetone, ethyl acetate, 30% methanolic solution of sodium methylate, methyl tert-butyl ether (MTBE), 2,6-di-tert-butyl-4-methyl phenol (BHT), gradient grade acetone, acetonitrile, chloroform, isopropanol, *n*-hexane, ethyl acetate and methanol for HPLC (Merck, Darmstadt, Germany) and reagents such as ethyl ether and petroleum ether (POCH, Gliwice, Poland), 99% ethyl alcohol (Chempur, Piekary Śl., Poland), citric acid, sodium sulphate, L(+)ascorbic acid, perchloric acid, BF_3 in methanol (Merck, Darmstadt, Germany), 1,1,3,3-tetrametoxypropane and 2-thiobarbituric acid from Sigma-Aldrich (St. Louis, MO, USA), silylating agent (HMDS, TMCS and pyridine at a 3:1:9 ratio) (Supelco, Bellefont, PA, USA) and KOH and NaCl (POCH, Gliwice, Poland) of analytical grade. Fatty acid standards were obtained from Larodan (Malmö, Sweden). Standards 7α- and 7β-hydroxycholesterol, 5,6α-epoxycholesterol, 5,6β-epoxycholesterol, 20α-hydroxycholesterol, cholestantriol, 7-ketocholesterol, 25-hydroxycholesterol, and 6-ketocholestanol as well as cholesterol, 5α-cholestan and α-tocopherol standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). Water deionized using a Milli-Qplus deionizer (Millipore, Vienna, Austria) was used for the analyses.

TABLE 1. Nutritive value of complete mixture and composition of fatty acids (% of total acids) in fat supplements.

Item	Mixture and fat supplements			
ME (MJ/kg)	13.6			
Crude protein (g/kg)	173			
Crude fat (g/kg)	40.1			
Lysine (g/kg)	10.0			
Methionine and cystine (g/kg)	59.0			
Tryptophan (g/kg)	20.0			
Threonine (g/kg)	62.0			
Ca (g/kg)	12.0			
P total (g/kg)	6.0			
CLA*	0.65			
Composition of fatty acids (% of total fatty acids)	Palm oil	Linseed oil	Rapeseed oil	Sunflower oil
SFA	45.13	11.28	7.46	8.48
UFA	54.87	88.72	92.54	91.51
MUFA	38.91	24.41	59.86	24.78
PUFA	15.96	64.30	32.68	66.74
PUFA *n-6*	15.13	27.20	25.12	66.19
PUFA *n-3*	0.51	36.84	5.95	0.30
CLA*	0.32	0.25	1.61	0.24

* tentative identification of CLA isomers

Analysis of fatty acids. The fatty acid profile of the oils and meat samples was determined using gas chromatography. The analysis was based on the Folch *et al*. method [1957], in which the sample was homogenised in a mixture of chloroform and methanol (v/v, 2/1), the solvent evaporated, and the evaporation residue saponified (0.5 N NaOH in methanol) and esterified (BF_3 in methanol). The fatty acid methyl esters produced were determined in hexane extracts using a Varian 3400 gas chromatograph (Sugar Land, TX, USA), a CP-Wax 58 capillary column packed with acid-modified polyethylene glycol, an 8200 CX injector and Varian-Star software for the processing and calculation of chromatograms.

Extraction of lipids from *m. longissimus dorsi* for analysis of cholesterol and PUC. Lipid extraction for determination of total cholesterol and its oxygen derivatives was performed using the method of Folch *et al.* [1957]. A ground sample (5 g) was weighed to an accuracy of 0.01 g and placed in centrifugation flasks, adding 250 μg of internal standard (6-ketocholestanol – IS, analysis of oxysterol; 5α-cholestan – IS, analysis of total cholesterol) and 5 mL of water at 35°C. This was supplemented with 50 mL of a chloroform and methanol mixture (2:1; v/v) containing 0.006% BHT and the mixture was homogenised for 3 min (8,000 rpm) using a DIAX 900 homogenizer (Heidolph, Germany). The sample was then shaken for 6 min and filtered through a paper filter (Filtrak 389). The filtrate was transferred into a conical separatory funnel, adding 15 mL of redistilled water. The lower chloroform layer was passed through anhydrous sodium sulphate (5 g) on a conical filter and the separatory funnel was additionally rinsed with 5 mL of chloroform. The chloroform fraction was collected into round-bottomed evaporation flasks (100 mL) into which 1 mL of anhydrous ethyl alcohol was added, and then evaporated to dryness using an R-200 rotary evaporator (Büchi, Flawil, Switzerland) in a nitrogen stream at a bath temperature of 40°C.

Analysis of cholesterol. To saponify a sample into previously evaporated samples, 20 mL of 1 N KOH in methanol was added. The mixture was shaken for 1 h at 40°C and left overnight at ambient temperature. After adding 20 mL of water, non-saponifying fractions were extracted using ether (25 mL). Ether fractions were rinsed 4 times with water, and the remainder was dissolved in 200 μL heptane and analysed using gas chromatography to determine cholesterol content.

Chromatographic analysis was performed using a Pro-GC gas chromatograph (Unicam, UK) equipped with a stream splitter and flame ionization detector (FID). Chromatographic separation was performed using a Chrompack WCOT Fused Silica capillary column (30 m, 0.25 mm, 0.25 μm) with a CP-Sil 8 CB stationary phase. The analysis was performed at a column temperature of 290°C and an injector temperature of 300°C. Helium was used as a carrier gas at a flow rate of 1.5 mL/min. A lipid extract or a mixture of standards (0.1-0.5 μL) was injected into the column using a 1:40 stream separator. Peaks were identified by comparison with standard retention times. The amount of cholesterol per sample was calculated by comparing the peak areas of cholesterol and 5α-cholestan (IS).

Analysis of oxysterols. Oxygenated derivatives of cholesterol were determined using a modified version of the method of Przygoński *et al.* [2000] based on the following modification: 2 mL of 10% sodium methanolate, mixed with methyl tertiary butyl ether (MTBE) at a 4:6 (v/v) ratio, was added to the lipid extract after evaporation of the solvent and transferred into 12 mL probes (Schott) equipped with a plastic screw cap and a PTFE seal and left for 1 h. Distilled water (4 mL) and then 5 mL of chloroform were gently added onto the probe walls. The sample was centrifuged to improve layer separation. Water addition was repeated 3–4 times until the chloroform solution became transparent. After removal of the aqueous phase, 2 mL of 1% aqueous citric acid solution were added to neutralize the alkaline environment and the sample was centrifuged again at 2,000 rpm. After removal of the aqueous phase, 1 mL of anhydrous ethanol was added to remove the remaining water. The chloroform phase was evaporated to dryness in a nitrogen stream at 30°C using a rotary evaporator.

Anhydrous sodium sulphate was placed on top of the Sep-Pak NH_2 column (Waters, Ireland) and conditioned with 5 mL of *n*-hexane. The esterified lipids were dissolved in 250 μL of chloroform and transferred into Sep-Pak. Non-polar matrix and cholesterol were eluted from the column using 5 mL of *n*-hexane, 5 mL of *n*-hexane/MTBE (5:1) and 5 mL of *n*-hexane/MTBE (3:1). The polar fraction (oxysterols) was eluted using 7 mL of acetone. The column purification process was performed using gravitational force at a flow rate of 0.3-0.5 mL/min. Acetone was evaporated in a nitrogen stream in a water bath at 30°C.

The dry remainder was supplemented with 75 μL of the mixture (HMDS, TMCS and pyridine at a 3:1:9 ratio) obtained from Supelco (Bellefonte, PA, USA) and transferred into chromatographic vials. The sample was closed under a stream of nitrogen and gently mixed. After 4 h, pyridine and the silylating agent were evaporated, and the dry remainder was dissolved in 100 μL of hexane. Under refrigeration conditions, silylol samples are stable for 48 h after preparation.

The calibration curve was plotted for six oxysterols (7α- and 7β-hydroxycholesterol; 5,6α-epoxycholesterol (α-epoxycholesterol), 5,6β-epoxycholesterol (β-epoxycholesterol), 20α-hydroxycholesterol, cholestantriol, 7-ketocholesterol and 25-hydroxycholesterol) obtained from Sigma-Aldrich (St. Louis, MO, USA). 6-ketocholestanol (200 μg/mL), added to the sample before extraction, was used as the external standard.

Chromatographic analysis was performed using a Unicam Pro-GC gas chromatograph (Cambridge, UK) equipped with a stream splitter and flame ionization detector (FID). Separation was performed using a Chrompack WCOT Fused Silica capillary column (30 m, 0.25 mm, 0.25 μm) with a CP-Sil 8 CB stationary phase at a programmed temperature of 130°C for 2 min, 25°C/min to 270°C, 2.5°C to 290°C, 12.6 min at 290°C. Helium flow was 0.6 mL/min. Splitless injection (1 μL) lasted 1 min.

Individual oxysterols were identified by comparing their retention times with those of oxysterol standards, and oxysterol content was estimated using the internal-standard method.

TBARS analysis. After 90 and 180 days of storage at -19°C, TBARS (secondary products of meat lipid oxidation, mainly malondialdehyde (MDA), which react with thiobarbituric acid) was determined in the samples of *m. longissimus*

dorsi according to a modified version of the method of Salih [1987] as modified by Pikul [1993].

Ground meat was weighed to a weight of 10.0 g, adding 34.25 mL cold (approx. 4°C) 4% perchloric acid and 0.75 mL alcohol butylhydroxytoluene solution, and homogenised for 2 min using a DIAX 900 homogenizer (Heidolph, Germany). The product was then filtered through a Whatman 1 filter and the filtrate was adjusted to 50 mL. After mixing, 5 mL of the supernatant was collected and transferred into 20 mL probes, adding 5 mL of 0.02-mol aqueous solution of 2-thiobarbituric acid (TBA reagent) and heated in a water bath in boiling water for 60 min. Absorbance was measured using a Beckman DU-640 spectrophotometer (Fullerton, USA) at a wavelength of 532 nm against the control sample containing 5 mL of 4% perchloric acid and 5 mL of TBA reagent. A calibration curve was plotted, using the solutions of 1,1,3,3-tetrametoxypropane at concentrations of 1×10^{-8} to 8×10^{-8} mol as a standard. The results were expressed as mg of malondialdehyde in kg^{-1} of fresh tissue.

Analysis of tocopherol. The content of vitamin E in *m. longissimus dorsi* was determined using a modified version of the method of Ueda & Igarashi [1987]. A 0.5-g portion of meat deprived of membranes and fascias was weighed to an accuracy of 0.0001 g into Schott glass vials (12 mL) and homogenised using a Moulinette® kitchen blender (Moulinex, France). This was followed by adding 1 mL of saturated solution of ascorbic acid in ethanol, 2 mL of KOH (60% m/v), 100 μL of NaCl (1%, m/v) and 100 μL of ethanol. After cooling, 100 μL of the internal standard (α-tocopherol), 100 μL of NaCl (1%, m/v) and 100 μL of ethanol were added. Vials were sealed and shaken using a Vortex shaker for approx. 10 s and then transferred to a water bath at 70°C, where the samples were saponified for 60 min. After cooling, 4.5 mL of 1% (m/v) NaCl solution was added, and 3 mL of an ethyl acetate and *n*-hexane mixture (1:9; v/v) was extracted by shaking (Vortex) the sealed probe for approx. 1 min. Then, 1.5 mL of supernatant was collected into chromatographic vials and evaporated to dryness under nitrogen gas in a water bath (40°C). The remainder was dissolved in 400 μL of ethanol and placed in the apparatus.

The determination was performed manually using an HPLC kit (Merck-Hitachi, Darmstadt, Germany) equipped with an L-7100 pump, L-7250 autosampler and FL L-7485 fluorescence detector (injection 40 μL; eluent – methanol + H_2O (96.5:3.5, v/v) (Lichrosolv, Merck); flow rate 1.0 mL/min (pressure approx. 160 atm.); time of analysis 27 min). Prior to analysis, the eluent was degassed using an ultrasound bath. Chromatographic separation was carried out using a LiChroCART™ 250-4 Superspher™ 100 RP-18 chromatographic column – 4 μm (Merck, Darmstadt, Germany). The data were integrated using HSM D-7000 LaChrom software (Merck-Hitachi, Darmstadt, Germany).

Statistical analysis. The results obtained were analysed statistically using two-way analysis of variance (ANOVA) and

TABLE 2. Fatty acids composition (the % of total fatty acids) in the m. *longissimus dorsi* of pigs (n=10) receiving different types of plant oils.

Fatty acids	Type of plant oil				Sex		SE	Interaction oil x sex
	Palm oil	Linseed oil	Rapeseed oil	Sunflower oil	gilt	barrow		
C 10:0	0,049	0.048	0.036	0.092	0.053	0.059	0.009	0.907
C 12:0	0.036	0.011	0.036	0.054	0.025	0.043	0.007	0.740
C 14:0	1.07	0.95	1.03	1.06	0.98	1.07	0.02	0.894
C 16:0	22.3	20.8	21.4	21.6	21.0 a	22.0^{b}	0.25	0.897
C 16:1 *n-7*	1.93	1.58	1.64	1.68	1.64	1.77	0.05	0.675
C 18:0	11.2	11.1	11.1	11.4	11.0	11.4	0.10	0.303
C 18:1 *n-9*	35.7^{b}	31.5^{a}	33.4^{ab}	34.1^{ab}	33.3	34.1	0.53	0.911
C 18:2 *n-6*	22.2^{a}	25.1^{b}	25.1^{b}	25.1^{b}	25.4	23.4	0.40	0.959
C γ18:3 *n-6*	0.15	0.15	0.16	0.13	0.15	0.14	0.005	0.622
C 18:3 *n-3*	1.10^{A}	4.69^{C}	2.17^{B}	1.12^{A}	2.35	2.19	0.24	0.981
C 20:0	0.11^{a}	0.11^{a}	0.11^{a}	0.13^{b}	0.11	0.11	0.002	0.461
C 20:4 *n-6*	2.63	2.20	2.33	2.09	2.49	2.13	0.12	0.840
C 20:5 *n-3* (EPA)	0.15^{aAB}	0.50^{cC}	0.25^{bB}	0.08^{aA}	0.26	0.23	0.027	0.956
C 22:6 *n-3* (DHA)	0.107^{b}	0.115^{b}	0.107^{b}	0.042^{a}	0.117^{B}	0.068^{A}	0.009	0.354
SFA	34.7	33.0	33.7	34.4	33.1^{a}	34.8^{b}	0.35	0.923
UFA	65.2	66.9	66.2	65.5	66.8^{b}	65.1^{a}	0.35	0.923
MUFA	37.6^{b}	33.1^{a}	35.0^{ab}	35.8^{ab}	34.9	35.8	0.58	0.895
PUFA	27.5	33.7	31.1	29.6	31.8	29.2	0.87	0.957
PUFA/SFA	0.80	1.03	0.93	0.86	0.97	0.84	0.03	0.946
PUFA *n-6*/PUFA *n-3*	18.4^{cC}	5.2^{aA}	11.2^{bB}	23.0^{dC}	14.3	14.7	1.19	0.688
Sum of CLA*	1.092	0.979	0.981	1.046	1.02	1.02	0.01	0.455

^{a,b} – values in the same rows with different letters differ significantly ($p<0.05$); ^{A,B} – values in the same rows with different capital letters differ highly significantly ($p<0.01$); * tentative identification of CLA isomers

Tukey's test. Interactions were determined between the experimental factors used, sex of animals and traits being determined, using Statgraphics 4.0 software. Data were tested at significance levels of $p<0.05$ and $p<0.01$.

RESULTS AND DISCUSSION

Feed mixtures are supplemented with vegetable oils to replace animal fats in livestock diets. This procedure enriches meat with UFA.

Table 2 presents the results of chromatographic analysis of the fatty acid profile of *m. longissimus dorsi*. Analysis of the fatty acid composition showed a significant difference ($p<0.05$) in MUFA levels between the groups receiving linseed oil and palm oil. This was affected by a higher level of oleic acid (C 18:1) in the group receiving supplemental palm oil. These results are corroborated by other studies, in which a positive effect of increased MUFA levels in pig feeding was obtained by Myer *et al.* [1992] for rapeseed oil and by Wiseman *et al.* [2000] for palm oil.

The effect of elevated MUFA levels in meat lipids on the quality of meat and processed products has been investigated in several studies. Rhee *et al*. [1988] reported that feeding pigs a 10% supplement of sunflower oil high in oleic acid for 90 days of fattening or a 12% supplement for 55 days doubled the MUFA to SFA ratio in intramuscular fat and in backfat compared to the control animals. The increased MUFA level in meat, when supplemental palm oil was used, did not reduce the quality of raw meat or processed products [Wiseman *et al.,* 2000]. In the present study, the level of linoleic acid (C18:2) decreased significantly in the group receiving palm oil compared to the other groups ($p<0.05$). Pork from pigs receiving dietary cereals is characterised by a high level of *n-6* linoleic acid (C18:2). Supplementation of the diets with MUFA in vegetable oils (sunflower oil and palm oil) and *n-3* PUFA, rich sources of which include linseed oil and rapeseed oil, results in significant changes in the fatty acid composition of carcass lipids, which is reflected in the physical traits of meat such as cohesiveness, firmness and tenderness. The use of dietary vegetable oils in the experiment induced differences in the level of *n-3* PUFA acids, including α-linolenic (C18:3), eicosapentaenoic (EPA) (C20:5) and docosahexaenoic (DHA) (C22:6) acids. The highest level of α-linolenic acid (C18:3) was found in the meat of the linseed oil-fed group compared to the other groups. Similar relationships were found for EPA and DHA acids, which were highly significantly more abundant in the groups receiving linseed oil and rapeseed oil compared to the groups supplemented with palm oil and sunflower oil ($p<0.01$). This effect was observed by other researchers [Barowicz & Pieszka, 2001; Myer *et al.,* 1992] who fed pigs supplemental linseed and/or rapeseed oil, which is indicative of the high conversion of *n-3* acids from feed to tissues. One hypothesis suggests that *n-3* and *n-6* acids compete in the activation of desaturases and in the synthesis of fatty acids. Moreover, the addition of fat sources rich in linolenic acid (rapeseed oil and linseed oil) and low in linoleic acid reduces the difference in the proportions of *n-6* and *n-3* acids, as confirmed by several pig feeding trials [Barowicz & Pieszka, 2001; Myer *et al.,* 1992], which plays a significant role in the prevention of coronary atherosclerosis in humans. In the present study, the *n-6* to *n-3* PUFA ratio was found to narrow highly significantly ($p<0.01$). In the groups receiving palm, linseed, rapeseed and sunflower oil, these values were 18.4, 5.2, 11.2 and 23.0, respectively. Significant differences were also found in the composition of fatty acids according to sex. The lipids of gilts showed a tendency towards a lower level of saturated acids and a higher level of unsaturated acids compared to the lipids of barrows ($p<0.05$). In addition, the DHA level was significantly higher in gilts than in barrows. These data are consistent with the findings of Warnants *et al.* [1999], who reported a higher level of saturated acids in barrows than in gilts. This fact was attributed to the higher activity of the enzyme acetyl-CoA-carboxylase (CBX) in barrows, reflected in the higher *de novo* synthesis of SFA and MUFA and the lower level of PUFA. This may be indicative of fatty acid metabolism following a different course according to sex [Hasty *et al.,* 2002]. The CBX enzyme is responsible for the first stage of fatty acid biosynthesis and plays a key role in the regulation of lipogenesis in pigs [Scott *et al*., 1981]. In order to avoid the negative impact of high-PUFA diets on pork quality, several suggestions have been made on the basis of many observations concerning the maximum level of PUFA in backfat and feed. It was suggested that feed should not contain more than 12–21% PUFA, and that in backfat this level should range from 12 to 22% [Warnants & van Oeckel, 1998]. The level suggested may also depend on whether meat has been processed, on storage conditions, on the antioxidants used and on the composition of major fatty acids. However, this level must be modified according to the composition of the major fatty acids, dietary antioxidants, duration of feeding, and the length of meat processing and storage periods.

The use of palm oil, linseed oil, rapeseed oil and sunflower oil supplements in diets in the second fattening period caused significant changes in the oxidative stability of meat, especially with longer storage periods. TBARS values for all the groups increased after 180 days of frozen storage of meat

TABLE 3. Effect of oil type in diet of fatteners (n=10) on level raw fat, oxidative processes and vitamin E status in m. *longissimus dorsi* after meat storage in -19°C.

Item	Type of oil				Sex		SE	Inter action oil x sex
	Palm oil	Linseed oil	Rapeseed oil	Sunflower oil	gilt	barrow		
Ether extract (%)	2.06	1.84	1.87	2.35	1.96	2.10	0.10	0.66
TBARS after 90 days of storage (mg/kg)	0.509	0.526	0.568	0.499	0.552^{b}	0.499^{a}	0.01	0.04
TBARS after 180 days of storage (mg/kg)	0.756^{A}	1.004^{B}	0.976^{B}	0.895^{AB}	0.895	0.921	0.02	0.003
α-Tocopherol (μg/g)	2.48^{bB}	1.72^{aA}	2.08^{abAB}	$1.91a^{AB}$	2.11	1.99	0.08	0.248

for explanations see Table 2

(Table 3). Highly significant differences were found in the TBARS content of meat between the group receiving palm oil and the group receiving rapeseed oil ($p<0.01$).

Similar results were obtained with rabbits by López-Bote *et al.* [1997], who suggest that vegetable oils rich in oleic acid (C 18:1), *e.g.* sunflower oil and palm oil, reduce lipid oxidation in meat. It was hypothesized that the degree of fatty acid oxidation depends on the degree of fatty acid unsaturation.

Some studies have shown that *n-3* fatty acids are particularly susceptible to oxidation. Hu *et al.* [1989] compared the susceptibility of the tissues of rats receiving diets rich in *n-3* or *n-6* PUFA acids to oxidation and found higher TBARS levels in the tissues of animals receiving higher levels of *n-3* acids. In our experiment, after 3 months of meat storage, the TBARS level was significantly lower in the meat of barrows compared to the meat of gilts ($p<0.05$). Similar results were obtained previously by Pieszka *et al.* [2004; 2006] and Hasty *et al.* [2002], who found a higher TBARS level in the meat of gilts, which can be attributed to the higher PUFA content of meat lipids and the higher susceptibility of these acids to oxidation. It seems interesting that the effect of sex was more pronounced in the susceptibility of lipids to oxidation in gilts after the first 3 months of frozen meat storage. After this period, a stronger effect was exerted by the type of fat supplement used. Regardless of the duration of storage, a significant interaction was observed between the type of fat supplement used, sex and TBARS level in meat ($p=0.04$ and $p=0.003$). In the present experiment, highly significant differences were found in vitamin E content of meat between the group receiving palm oil and the group receiving linseed oil ($p<0.01$). Similar findings were obtained for poultry by Sheehy *et al.* [1994] and for pigs by Daza *et al.* [2005], where a reduced vitamin E content in meat was observed as a result of using vegetable oil supplements in the diets. This mechanism is attributed to the use of tocopherols for stabilizing the double bonds of higher fatty acids found in cell membranes. Some authors reported lower levels of α-tocopherol in meat with the increasing dietary MUFA/PUFA ratio [López-Bote *et al.,* 2002]. However, other authors did not report this effect [Monahan *et al.,* 1992; Rey *et al.,* 2001a]. The role of vitamin E is to prevent lesions caused by oxidative factors by neutralizing free radicals in body cells. The use of higher PUFA levels in pig diets should account for increased levels of vitamin E in feed to prevent the oxidation of PUFA-rich meat fat and thereby increase its storage quality. Producers of meat products, especially cured meats, need fat that is stable, binds meat tissue well and is less susceptible to oxidation processes, which extends the shelf life of cured meats.

The 3% supplement of vegetable oils used did not cause any significant changes in cholesterol content of meat, although a certain tendency towards a lower cholesterol content was observed in the groups of pigs receiving rapeseed oil and linseed oil (Table 4). The total cholesterol content of *m. longissimus dorsi* ranges from 55 to 65 mg/100 g of fresh tissue [Maraschiello *et al.,* 1995], which is comparable with our results. The effect of dietary lipid composition on cholesterol content of meat is not clear. It is known that unsaturated acids found in the diet reduce the serum level of total cholesterol and LDL cholesterol in humans and animals. This effect results from the stimulation or inhibition of the activity of the enzyme that controls the cholesterol synthesis of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase in liver. Some believe that SFA activate the enzyme HMG CoA reductase responsible for cholesterol synthesis, while UFA reduce the level of HMG CoA reductase, but in our experiment we failed to observe this effect. Studies investigating the effect of feeding UFA to pigs on cholesterol content have been inconclusive. Some authors observed a tendency towards decreasing cholesterol levels in pig meat, while others failed to observe an effect of vegetable oils on the concentration of cholesterol in meat. Zanardi *et al.* [2000] reported that different dietary oils rich in MUFA or PUFA do not have an effect, while other authors [Barowicz *et al.,* 2000] have shown that *n-3* fatty acids have a hypocholesterolemic effect. Oxidized forms of cholesterol were found to form during storage. Highly significant differences were found in 7-ketocholesterol content between the groups receiving palm and sunflower oil and the groups receiving linseed and rapeseed oil ($p<0.01$). A similar highly significant correlation was shown for the level of total oxysterols and the level of total cholesterol, with differences between the group receiving linseed oil and rapeseed oil ($p<0.01$). The levels of 7α-hydroxycholes-

TABLE 4. Effect of oil type in diet of fatteners (n=10) on levels of cholesterol and oxysterols in m. *longissimus dorsi* after meat storage at -19°C.

Item	Type of oil				Sex		SE	Interaction oil x sex
	Palm oil	Linseed oil	Rapeseed oil	Sunflower oil	gilt	barrow		
Total cholesterol (mg/kg)	53.69	52.91	51.61	53.63	52.86	53.07	0.65	0.119
7α-Hydroxycholesterol (μg/g)	0.23	0.22	0.21	0.24	0.21	0.23	0.005	0.01
7β-Hydroxycholesterol (μg/g)	0.15	0.16	0.14	0.17	0.16	0.15	0.005	0.03
7-Ketocholesterol (μg/g)	1.73^{abA}	1.93^{cB}	1.83^{bcAB}	1.67^{aA}	1.81	1.77	0.02	0.10
α-Epoxycholesterol (μg/g)	0.16	0.16	0.15	0.18	0.16	0.16	0.006	0.03
β-Epoxycholesterol (μg/g)	0.18	0.19	0.17	0.21	0.19	0.18	0.007	0.03
20α-Hydroxycholesterol (μg/g)	nd	nd	nd	nd	nd	nd	nd	nd
25-Hydroxycholesterol (μg/g)	0.34	0.34	0.33	0.33	0.34	0.33	0.003	0.08
Cholestantriol (μg/g)	nd	nd	nd	nd	nd	nd	nd	nd
Total oxysterols (μg/g)	2.79	3.00	2.83	2.80	2.87	2.82	0.04	0.05
Proportion of cholesterol oxides to total cholesterol (%)	0.521^{A}	0.569^{C}	0.550^{B}	0.523^{A}	0.546^{B}	0.535^{A}	0.003	0.002

for explanations see Table 2, nd – not detectable

terol, 7β-hydroxycholesterol, α-epoxycholesterol, β-epoxycholesterol, 25-hydroxycholesterol and total oxysterols were observed to be highly significantly correlated to dietary ration and sex. These values ranged from p=0.002 to p=0.08. Eder *et al.* [2005] studied the effect of diets enriched with fatty acids and supplemental vitamin E on the level of oxysterols in meat and cured meats. A significant relationship was found between oxysterol content depending on the type of fat supplement used and the level of vitamin E in the diet. The meat of pigs receiving higher doses of vitamin E and palm oil in the diet was characterised by a lower degree of lipid oxidation (TBARS) and a lower oxysterol content in meat and meat products. Similar findings were reported by Eder *et al.* [2005], who fed pigs with supplemental soybean oil, palm oil and vitamin E and ascertained an inhibiting effect of the α-tocopherol supplement on the formation of oxysterols, especially in sausages produced from this pork. Based on a number of studies, it was found that fresh and briefly stored meat contains small amounts of oxysterols [Monahan *et al.,* 1992; Eder *et al.,* 2005], while in thermally-treated and refrigerated or frozen-stored meat and meat products, cholesterol can undergo considerable oxidation [Pie *et al.,* 1991; Rey *et al*., 2001b; Eder *et al.,* 2005]. A number of studies have shown that the use of antioxidants in the feeding of animals or as a supplement in meat products is one of the ways in which oxysterols are formed [Monahan *et al*., 1992; Eder *et al*., 2005]. It seems interesting that the levels of some oxysterols are highly significantly correlated to the fat supplement used and sex. It is concluded that the initiation of cholesterol oxidation in meat is affected by the composition of meat lipid fatty acids. In our experiment we observed that the more *n-3* PUFA the meat had, the more easily the cholesterol was oxidized.

CONCLUSIONS

In conclusion, there is a certain conflict between the dietetic and culinary value and the oxidative stability of pork enriched with PUFA. The introduction of *n-3* or *n-6* fatty acids into meat improves its dietetic value but may make the meat more susceptible to oxidation and all the other negative consequences associated with this process. The majority of secondary metabolites of fatty acid and cholesterol oxidation in meat are not neutral to our health and should be monitored to make food “safe”.

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WPŁYW DODATKU OLEJÓW ROŚLINNYCH DO DIETY TUCZNIKÓW NA UTLENIANIE LIPIDÓW ORAZ POWSTAWANIE UTLENIONYCH FORM CHOLESTEROLU W MIĘSIE

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Badano wpływ podawania tucznikom olejów roślinnych w diecie na stabilność oksydacyjną, poziom cholesterolu i powstawanie oksysteroli w mięsie. Doświadczenie wykonano na 40 tucznikach rasy pbz, podzielonych losowo na 4 grupy po 5 loszek i 5 wieprzków w grupie tuczonych od 50 do 105 kg masy ciała. Czynnikiem doświadczalnym były dodatki tłuszczowe: olej palmowy, olej lniany, olej rzepakowy i olej słonecznikowy podane w ilości 3% s.m. dawki pokarmowej. Stwierdzono istotnie wyższy poziom MUFA w mięśniu najdłuższym świń otrzymujących w dawce pokarmowej olej palmowy w porównaniu do grupy otrzymującej olej lniany ($p<0,05$),(tab. 2). Wykazano wysoko istotne zawężenie w proporcji kwasów z rodziny PUFA *n-6*/PUFA *n-3* pomiędzy grupami doświadczalnymi ($p<0,01$). Ponadto obserwowano istotnie wyższy poziom kwasu DHA u loszek w porównaniu do wieprzków ($p<0,01$). Zastosowanie dodatku olejów roślinnych w dawce pokarmowej spowodowało istotne obniżenie stabilności oksydacyjnej mięsa zwłaszcza po okresie 180 dni przechowywania mięsa w zamrożeniu ($p<0,01$), (tab. 3). Stwierdzono wysoko istotną interakcję pomiędzy zastosowanym dodatkiem tłuszczowym a płcią w zawartości TBARS ($p=0,003$). Wykazano wysoko istotne różnice w zawartości witaminy E w mięsie pomiędzy grupą otrzymującą olej palmowy a grupą otrzymującą olej lniany ($p<0,01$). Rodzaj zastosowanego oleju nie miał istotnego wpływu na zawartość cholesterolu ogólnego w mięsie. W trakcie przechowywania stwierdzono powstawanie utlenionych form cholesterolu. Wykazano wysoko istotne różnice w zawartości 7-ketocholesterolu pomiędzy grupami otrzymującymi olej palmowy i słonecznikowy a grupami otrzymującymi olej lniany i rzepakowy ($p<0,01$) (tab. 4). Podobną wysoko istotną zależność stwierdzono w poziomie sumy oksysteroli do zawartości cholesterolu całkowitego, przy czym zaznaczyły się również różnice pomiędzy grupą otrzymującą olej lniany i olej rzepakowy ($p<0,01$). Obserwowano wysoko istotne interakcje pomiędzy zawartością oksysteroli a zastosowanym źródłem tłuszczu i płcią, wartości te wahały się w zakresie $p=0,002$ do $p=0,08$ (tab. 4).