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ORIGINAL PAPER

Effect of dietary rapeseed oil and humus-containing mineral preparation on cholesterol and cholesterol oxidation products content in pork

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Abstract Two groups of finishing borrows (final crossbreeds of (Polish Landrace$_{(maternal)}$ × Large White Breed) × Pietrain$_{(paternal)}$) were fed a commercial feed (control group CG) enriched with either rapeseed oil or humus-containing mineral preparation (experimental group EG). Samples of tissue fat extracted from *Semimembranous* muscle contained an average of 12.95 mg/g cholesterol (CG) and 13.14 mg/g (EG). Used feed supplementation had significant effect on the inhibition of cholesterol oxidation process in pork. Sum of cholesterol oxidation products was lower in raw material of pigs fed enriched diet than in those fed only commercial diet. Additionally, cholesterol contained in raw material of finishers fed experimental diet was less susceptible to oxidation during storage. Results showed that samples of EG contain significantly ($p \leq 0.05$) less of such compounds as: 7α-hydroxycholesterol, 7β-hydroxycholesterol, 5,6α-epoxycholesterol, 5,6β-epoxycholesterol, 20α-hydroxycholesterol and cholestantriol in comparison with the control group. Results highlight the potential of using rapeseed oil and humus-containing mineral preparation to prevent cholesterol oxidation of pork.

Keywords Meat composition · Feed additives · Plant oils · Humic acid · Oxysterols

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Introduction

The modern consumer, aware of the relationship between eating habits and health, more and more attention directed at the safety, nutritional and dietary value of purchased food. Producers of foodstuffs to meet the expectations of consumers, they are forced to look for methods of food production with the desired properties, corresponding to the nutritional recommendations, while maintaining the health safety throughout the whole production chain. Food modifications related to increase the health benefits of its consumption depend on increasing the content of nutritionally desirable ingredients and reducing those that may have a negative effect on human health and well-being [1].

In recent decades, much attention is paid to the compounds accompanying the lipids of food of animal origin—cholesterol and its derivatives. Cholesterol is an important molecule that has many biological functions, e.g., is a part of the myelin sheath of nerve cells, is a precursor of bile acids, adrenal steroid hormones, and sex hormones and also participates in the synthesis of vitamin D [2]. Most of total cholesterol contained in the human body, as well as in animal body, is endogenous, mainly synthesized by the liver, but some is absorbed from dietary sources [3]. Cholesterol, due to the presence of the double bond, may undergo oxidation processes [4]. The oxygenated derivatives (known as oxysterols, cholesterol oxidation products—COPs) demonstrated more potent properties of atherosclerotic and carcinogenic and mutagenic than cholesterol, which arise [5, 6]. The major source of cholesterol in human diet is animal products such as meat, eggs, fish, poultry and milk fat. Its bioavailability increases while cooking process, but at the same time high temperatures may lead to its undesirable modifications, i.e., formation of oxides [3]. Most of

Table 1 Chemical composition of 1 kg basal diet

	35–50 kg body weight	50–110 kg body weight
Energy (kcal)	3128	3105
Crude protein (%)	15.9	15.0
Crude fiber (%)	5.0	5.0
Total phosphorus (%)	0.63	0.63
Dicalcium phosphate (%)	0.75	0.72
Lysine (%)	0.93	0.78
Met +Cys (%)	0.67	0.68
Threonine (%)	0.59	0.56
Tryptophan (%)	0.19	0.18
Vitamin A (UI)	8000	5000
Vitamin D_3 (UI)	1390	870
Vitamin E (UI)	91	60

oxysterols contained in animal food products are probably generated in a non-enzymatic way during cooking, processing and storage [7]. Thus, foods processed from cholesterol-rich raw materials may be the greatest source of COPs found in the human body [8]. Relatively high proportion of fat, cholesterol and iron in pork results in its enhanced susceptibility to oxidation process. Moreover, oxidized lipids, which are formed from highly unsaturated fatty acids, may affect the oxidation of associated cholesterol, and thus pork may serve as a potential source of oxysterols [9–11]. With attention to above reports, it is highly important to monitor the occurrence of COPs in meat and meat products with accurate and sensitive procedures [12]. The objective of presented study was to determine the effect of fodder enrichment with rapeseed oil and humus-containing mineral preparation on cholesterol and cholesterol oxidation products content in fresh and storage pork cuts.

Materials and methods

Animals and diet

The study was conducted on borrows (final cross-breeds of (Polish $\text{Landrace}_{(\text{maternal})}$ × Large White Breed) × $\text{Pietrain}_{(\text{paternal})}$). Rearing of pigs was introduced in stock farm located in central western Poland. Pigs were divided into two groups of 25 in each—control (CG) and experimental group (EG). In feeding of both groups was used a complete standard diet applied in two growth phases (Table 1). Additionally, the diet of experimental group was enriched in Humokarbowit® (humus-containing mineral preparation, 4 % of complete feed, TRONINA Innovative Breeding Company Stanislaw Tronina, Poland) and rapeseed oil (10 g kg^{-1} added in second phase of fattening). Rapeseed oil was used as a carrier of polyunsaturated fatty acids, in particular n-3 fatty acids and vitamins E of antioxidant properties [13]. Humokarbowit® is a feed additive prepared from humic brown coal, peat and feeding dolomite and contains: 30.5 % natural humic acids, 1.95 % bitumen, 1.16 % nitrogen, 5.50 % calcium, 2.95 % magnesium, 1.90 % silicon, 1.00 % sulfur and 0.55 % aluminum. This humus-containing mineral preparation increases the biological value of feed, showing bactericidal and fungicidal properties, also used in pig nutrition positive influence on digestive (prevents diarrhea), stimulate immunity of animals, as well as have a positive influence the quality of animal raw materials [14–16]. Pigs were slaughtered with an average live weight of 110 kg in Meat Plant Salus (Golinka, Poland). The meatiness of carcasses under investigation amounted to 58 %. The experimental material—*Semimembranous* muscle—was collected from the right side of carcasses and delivered to Department of Animal Products Technology and Quality Management on Wroclaw University of Environmental and Life Sciences (Poland) after 24-h *postmortem*. Samples of the muscles (app. 250 g) were packaged in polyethylene bags for frozen storage and were hold in 2–3 °C for analysis in fresh material and for 6 months in −18 ± 0.5 °C for analyses in stored material.

Chemical analysis

Total fat content was analyzed according to procedures described in Polish Standard PN-ISO 1444:2000 [17]. The analysis was conducted in triplicate.

Lipids for fatty acids, cholesterol and cholesterol oxidation products (COPs) analyses were extracted with standard procedure [18] using methylene chloride and methanol (2:1).

Preparation of methyl esters of fatty acids was conducted in accordance with PN-EN ISO 5509: 2001 [19]. Chromatographic analysis was performed using a gas chromatograph coupled with a spectroscopy mass detector (Agilent 6890 N Series, 5973 MS). Separation of fatty acids methyl esters was carried out using chromatograph column DB-225MS (60 m, 250 µm; 0.25 µm). Samples of 1 µl were transferred to the column with the carrier gas (He), split 1:100. Temperatures of column and injector were set at 160 and 280 °C, respectively. Oven temperature was programmed as follows: 140 °C for 5 min., 4 °C/min to 240 °C. Peak identification was made by comparing the retention times with commercial standards (FAME Mix, Sigma–Aldrich Inc., St. Louis, MO, USA).

Analysis of cholesterol was performed according to method described by Kovacs et al. [20]. Chromatographic analysis was carried out using Unicam ProGC equipped

with a flow divider and flame ionizator detector (FID). Analysis was carried out on capillary column Chrompack WCOT Fused silica (30 m; 0.25 mm; 0.25 μm) with the stationary phase: CP-Sil 8 CB. Helium was used as the carrier gas, the split ratio was 1:40, the sample volume injected was 1 μl. Temperatures of column and injector were set at 290 and 300 °C, respectively. Oven temperature was programmed from 160 °C for 1 min, 40 °C/min to 270 °C, 4 °C/min to 280 °C (held for 15 min). Identification of the peaks was made by comparing the retention times with commercial standard—5α-cholestane (Sigma–Aldrich Inc., St. Louis, MO, USA). The quantification of cholesterol in the sample was calculated by comparing the peak areas of cholesterol and 5α-cholestane.

Analysis of cholesterol oxidation products was performed by a modified method given by Schmarr et al. [21]. Calibration curve was plotted for eight oxysterols (7α-hydroxycholesterol; 7β-hydroxycholesterol; 5,6α-epoxycholesterol; 5,6β-epoxycholesterol, 20α-hydroxycholesterol; cholestantriol, 7-ketocholesterol, 25-hydroxycholesterol) purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Chromatographic analysis was done by using the same equipment and conditions as reported for the analysis of cholesterol. The individual oxysterols were identified by comparing the retention times with their commercial standards, and quantification was carried out by comparing the peak areas of particular COPs and internal standard (5α-cholestane).

Data were analyzed statistically using Statistica software ver. 8.0. (StatSoft Inc., Krakow, Poland). Statistically significant effects were identified using Duncan's multiple range test ($p \leq 0.05$). The results were presented as means of replicates and standard error (mean ± standard error).

Results and discussion

The result of rapeseed oil addition on the fatty acids content in the fodder was easy to predict. Rapeseed oil, used as a source of polyunsaturated fatty acids, significantly ($p \leq 0.05$) increased their content in the diet (Table 2). Oil addition resulted especially in the changes of n-3 PUFA content and ratio of n-6 to n-3 polyunsaturated fatty acids. Rapeseed oil increased almost two times share of the n-3 group and at the same time decreased the ratio of n-6 to n-3 from 8.6 in CG diet to 4.8 in EG diet. Also, after storage the content of polyunsaturated fatty acids in experimental diet was higher than in the diet of the control group.

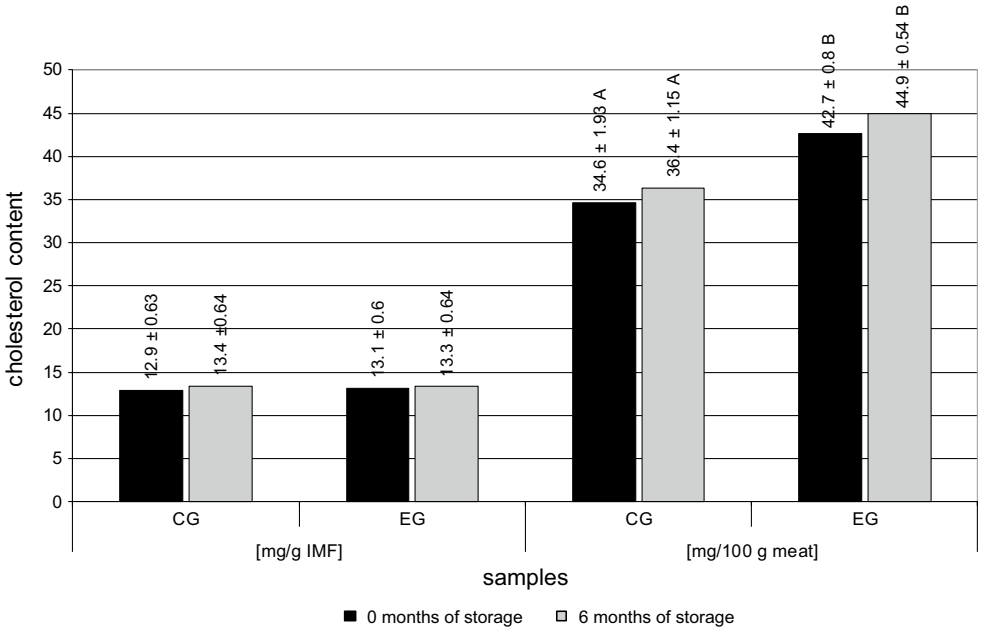
The analysis of collected data (Fig. 1) indicated a lack of variation in the total cholesterol content. Samples of tissue fat contained an average of 12.95 mg/g cholesterol in case of control group and 13.14 mg/g in experimental group. Frozen storage of raw meat for a period of 6 months did not have a significant ($p \geq 0.05$) impact on the concentration of this compound. However, expressing the content of cholesterol per 100 g of meat provides more differentiation depending on the fat content in meat samples. In present study, modification of diet affected a significant ($p \leq 0.05$) increase of fat content—raw material collected from EG group contain more of this component compared with the control group (respectively: 3.25, 2.67 %) and as the same time meat from experimental group characterized by higher cholesterol content in comparison with the control group. Fresh and storage hams of experimental group contained an average 8 mg more cholesterol (42.71 and 44.92 mg/100 g, respectively) than control group (34.59 and 36.38 mg/100 g, respectively), which indicates the ability to deliver a larger amount of cholesterol, together with the consumption of meat richer in fat.

Table 2 Fatty acids content in fresh and storage diet (% of total FA)

Group fatty acids	Storage time (weeks)	Diet of CG	Diet of EG
SFA	0	22.32 ± 0.06^{b}	20.28 ± 0.29^{a}
	16	26.07 ± 0.06^{b}	21.30 ± 0.43^{a}
MUFA	0	31.93 ± 0.08^{b}	31.47 ± 0.08^{a}
	16	30.97 ± 0.23^{a}	31.36 ± 0.12^{a}
PUFA	0	45.74 ± 0.56^{b}	48.24 ± 0.09^{b}
	16	42.95 ± 0.40^{a}	47.31 ± 0.53^{b}
n-3 PUFA	0	4.76 ± 0.03^{a}	8.29 ± 0.01^{b}
	16	3.17 ± 0.20^{a}	7.99 ± 0.10^{b}
n-6 PUFA	0	40.96 ± 0.23^{b}	39.95 ± 0.09^{a}
	16	39.78 ± 0.60^{a}	39.29 ± 0.90^{a}
n-6/n-3 Ratio	0	8.60^{b}	4.82^{a}
	16	12.55^{b}	4.92^{a}

[a,b] Different letters in the row indicate significant differences ($p \leq 0.05$) between values

Dietary manipulations leading to changes in the fatty acid profile of pork fat are improving the quality of pork for the consumer and meeting nutritionists' recommendation [22]. On the other hand to high level of polyunsaturated fatty acids may increase the susceptibility to lipid oxidation [23]. Oxidation products of PUFAs accelerate the formation of oxidized forms of cholesterol in the long-stored meat. In view of the literature, the content of oxysterols in fresh meat is insignificant, while the processes such as storing or heat treatment will increase the content of these compounds [24–26]. As a result of the analysis (Fig. 2), it has been shown that through modification of borrows' diet, it was possible to lower the content of cholesterol oxidation products. The fat extracted from the meat of pigs fed supplemented fodder characterized by significantly ($p \leq 0.05$) lower sum of cholesterol oxidation products (19.72 μg/g). Intensive process of cholesterol oxidation was observed



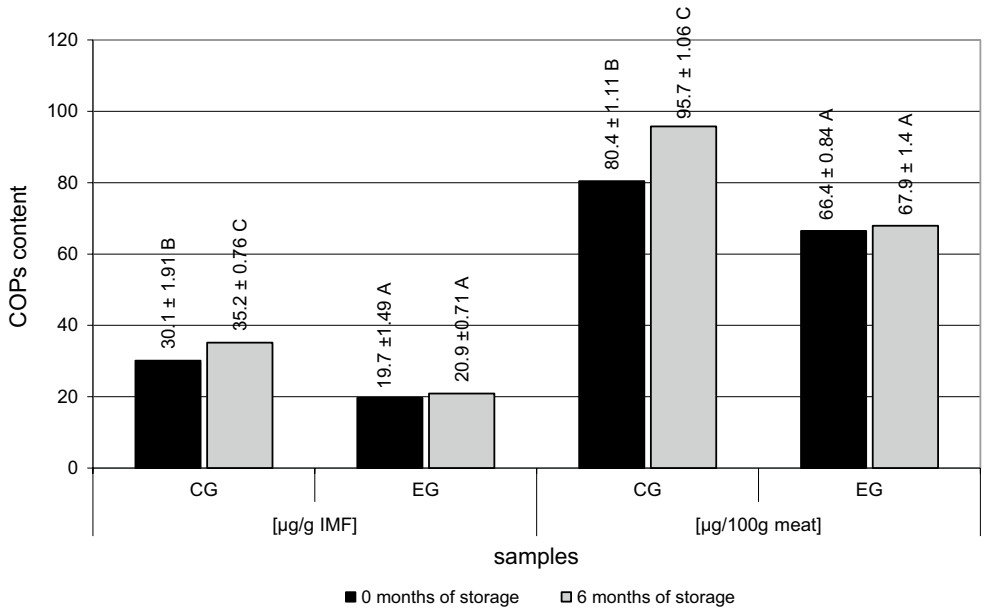


Fig. 1 Cholesterol content of fresh and storage meat samples, $n = 30$. *A, B* Different letters indicate significant differences ($p \leq 0.05$) between values

Fig. 2 Sum of cholesterol oxidation products, $n = 30$. *A, B, C* Different letters indicate significant differences ($p \leq 0.05$) between values

in samples of control groups where the sum of COPs was the highest (30.12 µg/g). In addition, the storage process caused an increase in COPs amount of further 5.08 µg/g of fat. It is worth noting that cholesterol contained in raw material of finishers fed experimental diet was less susceptible to oxidation during storage; that is, amount of cholesterol oxidation products in storage meat of EG group was negligible. Therefore, expressing the content of cholesterol oxidation products per 100 g of meat, it was found that the meat of the control group contained an average of 80.41 µg COPs in 100 g of meat, while in the meat samples of experimental group have been marked by almost 14 µg less of these compounds.

Dietary trends of rising PUFAs in the diet also emphasize the desirability of supplementing the diet of farm animals in compounds with antioxidant properties [27, 28]. Observed in presented study, restricted accumulation of oxysterols in meat might be due to the fact that rapeseed oil is a rich source of tocopherols, which are efficient scavengers of free radicals and prevent lipid peroxidation [29].

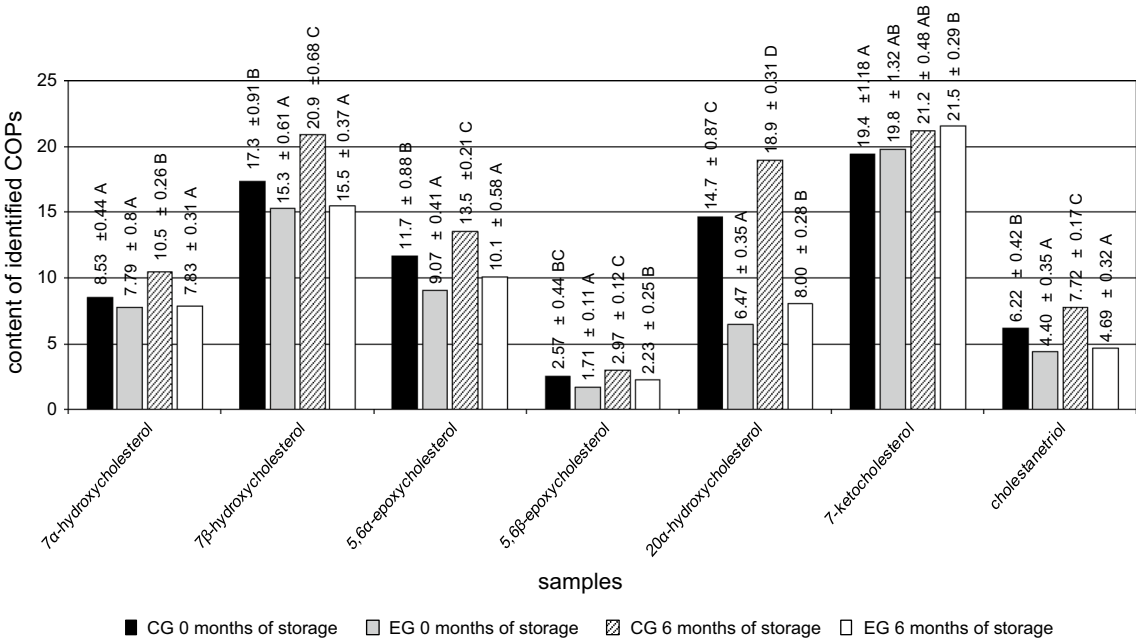


Fig. 3 Content of identified cholesterol oxidation products (μg/100 g of meat), $n = 30$. *A, B, C, D* Different letters indicate significant differences ($p \leq 0.05$) between values

The presence of humic substances in the experimental diet also might affect the content of cholesterol oxidation products. Humic substances can form complexes with metal ions, oxides, clay minerals and can interact with organic compounds, e.g., fatty acids [14].

Results of present study confirmed that fodder supplementation with rapeseed oil had an effect on the amount of identified oxysterols in meat samples (Fig. 3). In comparison with the control group, samples of experimental material contain significantly ($p \leq 0.05$) less of such compounds as: 7α-hydroxycholesterol, 7β-hydroxycholesterol, 5,6α-epoxycholesterol, 5,6β-epoxycholesterol, 20α-hydroxycholesterol and cholestantriol. The level of oxidation in meat products depends on the quality of the raw materials, the amount of added substances with antioxidant properties, the processing conditions and the length of ripening and storage [3]. Conchillo with co-workers [30] demonstrated that amounts of 7α-hydroxycholesterol, 7β-hydroxycholesterol, 25-hydroxycholesterol and β-epoxycholesterol depend on the type of packaging during storage and were lower in vacuum-stored raw chicken meat than in aerobically stored samples, probably due to the lack of oxygen under vacuum, which would protect cholesterol from oxidation. The content of 7-ketocholesterol in pork samples of both feeding groups of pigs developed on approximately the same level (19.4 μg/100 g of meat in control group and 19.8 μg/100 g of meat in experimental group, respectively). 7-ketocholesterol is a common indicator to measure oxidation of food of animal origin [31]. Presented results are much lower than obtained by Lercker and Rodriguez-Estrada [32] who stated that raw meat presented a considerably high initial 7-ketocholesterol level (3.5 ppm), as a direct consequence of the holding period in order to increase the meat tenderness and to promote the flavor formation. Also Baggio et al. [33] reported lower concentration of this COP, i.e., 330 μg/kg sample in raw turkey breast meats after 16 month of frozen storage. Broncano et al. [31] indicated lack of 7-ketocholesterol in raw meat (*Latissimus dorsi*) of Iberian pigs. In contrast, investigation of COPs concentration in *Latissimus dorsi* of Iberian pigs showed high level of 25-hydroxycholesterol (22.1 μg/100 g); however, in our study none of analyzed pork samples had detectable concentrations of this compound.

Conclusion

In conclusion, our study showed that addition of humus-containing mineral preparation and rapeseed oil to pig's diet decreased cholesterol oxidation in pork. The meat samples of control group contained larger amounts of harmful COPs, especially β forms, and in particular strongly mutagenic cholestanetriol, than material collected from experimental group. Based on the results obtained in this study, it is possible to apply rapeseed oil to the fodder as a carrier of polyunsaturated acids without negative effects on cholesterol's stability in stored meat.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements All applicable national, and institutional guidelines for the care and use of animals were followed.

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