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The effect of diet supplementation with Se, Zn and vitamin E on cholesterol, CLA and fatty acid contents of meat and liver of lambs*

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The study was conducted on 20 Polish Merino ram-lambs divided into two groups: experimental (E) and control (C), each of 10 animals. From birth all the lambs were maintained with their dams and then weaned at the age of 8 weeks, placed in individual straw-bedded pens and fattened individually with a granulated concentrate mixture until reaching a mean body weight of 31.8 kg. During fattening each lamb from group E was given *per os* 1 ml 0.1% Na_2SeO_4, 3 ml 10% $ZnSO_4$ and 60 mg vitamin E (α-tocopherol) daily. The concentrations of conjugated linoleic acid (% of the fatty acid sum) in meat (0.45%) and in liver (1.21%) were higher (P<0.05) while cholesterol content (42.8 mg/100g fresh matter) lower (P<0.05) in E than in C lambs. In group E total cholesterol of blood plasma decreased and HDL fraction level increased. The dietary supplementation of Se, Zn and vitamin E improved the lipid profile of lambs' meat.

KEY WORDS: cholesterol / CLA isomers / lambs / selenium / vitamin E / zinc

One of the purposes of modifying animal fats is to produce high quality products meeting dietary recommendations for a reduced intake of saturated fatty acids (SFA) and an increased intake of mono- (MUFA), and polyunsaturated (PUFA) fatty acids

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in the human diet. Consuming such products lowers risk of obesity, cancer, diabetes and cardiovascular diseases [Grundy 1999, Voigt and Hagemeister 2001]. Conjugated linoleic acid (CLA) isomers found in ruminant's milk and meat are by-products of incomplete biohydrogenation of lipids by ruminal bacteria *Butyrivibrio fibrisolvens* [Kepler *et al*. 1966].

CLA refers to a mixture of positional and geometric isomers of octadecadienoic (linoleic) acid with conjugated double bonds. The most extensively investigated CLA isomer is *cis-9,trans-11* octadecadienoic acid, which is thought to be biologically active [Chin *et al*. 1992]. Ruminant meat generally contains more CLA than meat from non-ruminants [Bessa *et al*. 2000]. Traditionally meat occupies a special place in the diet because of its appealing flavour and texture and its high nutritive value. Some of the main factors limiting the quality and acceptability of meat are lipid oxidation products and concentration of cholesterol, SFA, PUFA and CLA [Morrissey *et al*. 1998].

Se is implicated in antioxidant functions, and Se-Cys complex is essential in the active centres of Se-enzymes that carry out redox reactions, glutathione peroxidase (GPx), thyroid hormone deiodinase families and thioredoxinreductase [Tapiero *et al*. 2003]. Vitamin E is primarily active as an antioxidant protecting PUFA *in vivo* and *post-mortem* in animal tissues and muscle nutrients from free-radical attack [Morrissey *et al*. 1994]. Dietary supplementation of vitamin E increases the concentration of α-tocopherol in muscle and reduces the susceptibility of the muscle to lipid oxidation [Buckley *et al*. 1995]. Zinc is associated with enzymes, both as part of the molecule and as an activator. In its structural role, Zn usually stabilizes the structures of RNA, DNA, and ribosomes [McDowell 1992]. Zn superoxide dismutase (ZnSOD) is a dietary factor which contributes to the antioxidant defence system [Morrissey *et al*. 1998]. In several studies using rats it was found that the content of PUFA, especially in serum cholesterol esters and phospholipids was positively associated with Se concentration in the diet [Crespo *et al*. 1995]. Czauderna *et al*. [2003] suggested that the interaction between Se and the CLA isomers mixture protected CLA from peroxidation damage in muscles and increased the level of CLA in the muscles of rats.

The aim of the present experiment was to test the hypothesis that addition of Se, Zn and vitamin E to the diet for fattening lambs results in decreasing the cholesterol level and increasing the PUFA and CLA levels of meat.

Material and methods

The experiment was carried out on 20 Polish Merino ram-lambs divided into two groups of 10: control (C) and experimental (E). Till the age of 8 weeks all the lambs were maintained with their dams. Then the lambs were weaned, placed in individual straw-bedded pens and fattened individually with a pelleted concentrate mixture (Tab. 1) offered *ad lib*. until the animals' mean live weight of 31.8 kg. Mean initial body

weight of lambs (groups pooled) was 18.9 kg (SE = 0.9), fattening lasted 42.1 days (SE = 2.9) and daily live weight gain during fattening was 317.4 g (SE =13.2).

Additionally, about 100 g hay per lamb/day was offered. The hay was not fully consumed and its intake was not recorded. During fattening period the daily amount of concentrate consumed was recorded individually. Proximate analysis of feeds was performed using standard methods. The level of metabolizable energy of feed was calculated on the basis of the results of proximate analyses using the equation recommended by MAFF [1975].

During fattening, each lamb from group E was given *per os* 1 ml 0.1% Na_2SeO_4, 3 ml 10% $ZnSO_4$ and 60 mg vitamin E (α-tocopherol) daily. The content of Se and Zn in the concentrate was 0.15 and 28.8 mg/kg DM, respectively. The loin (*longissimus dorsi* muscle) and liver samples were taken after slaughter to determine the concentrations of SFA, MUFA, PUFA and CLA. For determination of cholesterol in meat the total lipid fraction was extracted with chloroform-methanol mixture (2:1, v/v) after Folch *et al*. [1957]. Total cholesterol was determined colorimetrically according to Searcy and Berquist [1960].

For determination of fatty acids the tissue samples were freeze-dried and extracted with chloroform-methanol-water mixture (4:2:1.v/v). Hydrolization and derivatization reaction was carried out according to Czauderna *at al*. [2001]. The derivatized samples were filtered through 0.2 μm membrane filter (WHATMAN). The filtrates were injected onto chromatographic column Spheri-5 RP-18.5 μm, 220 × 4.6 mm (PERKIN ELMER). The CLA isomer mixture standard (the *cis-9,trans-11* and *trans-10,cis-12* CLA) and other fatty acid standards were provided by SIGMA. The blood was withdrawn at the end of fattening. The total and HDL (high-density lipoprotein) cholesterol and triglycerides (TG) were determined in blood plasma using enzyme-linked test kits from Alpha Diagnostic S.A. (Warsaw, Poland).

The Zn content in feeds was determined with atomic absorption spectrometry. Samples (0.5 g) were mineralized in a mixture of 5 ml HNO_3 and 1 ml H_2O_2 in hermetic high-pressure vessels by heating in microwave oven.

Total Se content was estimated with the flame (air-acetylene) atomic absorption spectrometry (PERKIN ELMER 1100B) using hydrogen generation system. Selenium hydride was generated with $NaBH_4$ (3% solution in 1% NaOH). Hallow cathode lamp (196.0 nm) with deuterium background correction was used.

Means and their standard errors were computed and differences between group means verified based on the t-test using Microsoft EXCEL and STATISTICA for Windows.

Results and discussion

Feed ingredients of concentrate mixture are shown in Table 1, while Table 2 shows the results of proximate analyses of both feeds and their nutritive value indicators, as well as Zn and Se content of dry matter. Daily intake of dietary DM (groups pooled)

Table 1. Composition of concentrate mixture

Ingredient	Per cent
Ground barley	44.1
Ground oats	17.0
Ground field bean	10.0
Rapeseed oilmeal	25.0
Minerals	2.9
Beet molasses	1.0

Table 2. Chemical composition and nutritive value of feeds (g/kg DM)

Component	Concentrate mixture	Meadow hay
Dry mater (%)	871	850
In dry mater		
organic matter	924	933
crude ash	76	67
crude protein	205	121
crude fibre	76	315
ether extract	52	18
N-free extractives	595	470
NDF	209	634
ADF	109	365
ADL	35	42
Metabolizable energy (MJ/kg)	12.5	10.2
Se (mg/kg DM)	0.15	0.10
Zn (mg/kg DM)	28.8	15.1

was 0.97±0.2 kg from concentrate and 0.085 kg from hay (figures not tabulated). Daily Se intake in C group was 0.15 mg and 0.57 mg in group E while the respective values for Zn were 29.2 mg and 97.2 mg. Minimum dietary Se requirements of animal species cannot be given with any accuracy. However, the National Research Council (USA) suggests that requirements for Se of sheep range from 0.1 to 0.2 ppm of feed DM [McDowell 1992]. Some animal experiments have suggested that dietary Se may have certain beneficial effects at levels higher than those generally accepted as adequate [McDowell 1992].

Current estimates put maximum tolerable level of Se at 2 mg/kg DM for the major livestock species, no differences being indicated between ruminants and non-ruminants [McDowell 1992, Davis *et al*. 2006]. The maximum tolerable level of inorganic Se for sheep is much higher than 2 mg/kg DM as was suggested earlier. Feeding up to 12 mg of selenite/kg feed DM to ewes under the stress of production (*i.e*. gestation and lactation) for 72 weeks did not produce any pathological manifestations

of Se intoxication [Davis *et al*. 2006]. The NRC suggested that requirements for Zn of sheep vary from 20 to 33 mg/kg feed DM. For ruminants, overt Zn toxicosis first appears when levels around 1000 ppm are incorporated into a natural-ingredient diet [McDowell 1992].

In our earlier study on lambs the supplementation of Zn, Se and vitamin E led to decrease of fat content of leg (P≤0.05) and fat thickness over the loin eye and over ribs as compared to controls [Gabryszuk *et al*. 2003]. Lambs fed diets with higher levels of Zn, Se and vitamin E showed increase in Se and vitamin E concentration of loin, not however, accompanied with the increase in Zn content [Gabryszuk *et al*. 2005a]. On the other hand, the Se, Zn and vitamin E intra-muscular injections of ewes after lambing were effective in increasing both minerals and the vitamin contents of milk [Gabryszuk *et al*. 2005b].

In comparison to the C diet, enrichment of diet E with Se, Zn and vitamin E (0.42, 68 and 60 mg /lamb/day, respectively) significantly decreased the cholesterol content

Table 3. Total cholesterol content of loin (fresh tissue) and total cholesterol, HDL and triglyceride (TG) of blood plasma in lambs

Item	Group E		Group C	
	mean	SE	mean	SE
Loin				
cholesterol (mg/100 g)	42.8[a]	33.8	72.0[b]	24.1
Blood plasma				
total cholesterol (mmol/l)	2.53[a]	0.20	2.75[b]	0.17
HDL (mmol/l)	0.84[a]	0.07	0.77[b]	0.07
TG (mmol/l)	0.42	0.03	0.43	0.05

[ab]Within rows means bearing different superscripts differ significantly at P≤0.05.

Table 4. Fatty acid composition (% of total fatty acids) of loin and liver of lambs

Item	Group E		Group C	
	mean	CV	mean	CV
Loin				
SFA (%)	38.3	11.5	37.5	23.7
MUFA (%)	42.6	10.6	43.2	28.9
PUFA (%)	19.1	9.6	19.3	28.6
CLA (%)	0.45[a]	33.5	0.34[b]	53.4
Liver				
SFA (%)	40.2	8.4	39.5	8.0
MUFA (%)	37.7	16.6	38.3	14.1
PUFA (%)	22.1	12.4	22.2	11.8
CLA (%)	1.21[a]	26.6	0.93[b]	20.0

[ab]Within rows means bearing different superscripts differ significantly at P≤0.05.
SFA: C3:0, C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0 and C18:0
MUFA: C10:1, C12:1, C14:1, C16:1, C18:1
PUFA: C18:2, C18:3, C20:3, C20:4, C20:5
CLA: a sum of CLA isomers: *cis*-9,*trans-11* CLA and *trans-10,cis-12* CLA.

of loin and the level of total cholesterol of blood plasma with simultaneously lower HDL level (Tab. 3). The content of triglycerides (TG) of blood was similar in both groups (Tab. 3) while the concentration of CLA of loin and liver was higher in group E (Tab. 4).

Falkowska *et al*. [2000] showed that supplementation of a diet with Se and vitamin E significantly increased HDL fraction in blood of cows – from 0.440 to 0.552 mmol/l. Brzóska and Brzóska [2004] observed that when the level of dietary Se increased from 0.04 to 0.48 mg/kg DM, the cholesterol content of blood plasma of cows decreased from 228.6 to 183.9 mg/dl, with simultaneous decrease in HDL and LDL fractions. The increased concentration of HDL in blood plasma may stimulate the mammary gland to increase the content of UFAs in milk [Brzóska and Kowalczyk 2002]. On the other hand, long-chain fatty acids are known to decrease the LDL content of liver, which at their declining tendency in blood plasma, could promote the increased HDL content of plasma [Brzóska and Kowalczyk 2002]. Borys *et al*. [2004] reported that supplementing lambs' diet with oilseed and 224 mg/kg feed vitamin E led to the increased level of the latter as well as of PUFA Ω3 acids and CLA in meat. The concentration of CLA increased from 0.23 to 0.58% of the sum of fatty acids. It is worth to note a trend for reduced cholesterol content in the muscles of all experimental lambs in relation to the controls [Borys *et al*. 2004]. Szumacher-Strabel *et al.* [2001] reported that lambs fed a diet with rapeseed oil, linseed oil or hydrogenated rapeseed oil showed the meat CLA content on the level 0.02-0.03 μg/g tissue. The diet enriched with Se (2 ppm feed) led to the increased, however not significantly, content of CLA isomers (from 1.04 to 1.73 mg/g) of the fresh liver [Czauderna *et al*. 2004a].

Czauderna *et al*. [2003] suggest that the diets enriched in Se and individual CLA isomers, or their mixture, stimulate lipogenesis, desaturation and elongation as well as inhibit fatty acid β-oxidation in muscles of rats. They found that Se and individual CLA isomers or their mixtures fed to rats led to increase the level of CLA, *cis*-MUFA and PUFA in the muscles. Crespo *et al*. [1995] reported that the concentration of PUFA was positively correlated with the level of Se in diets of rats. The *cis-9*,*trans-11* CLA found in ruminant milk and meat is an intermediate in the biohydrogenation of linoleic (*cis-9*,cis-*12* C18:2) to stearic acid (C18:0) – Bessa *et al*. [2000]. The major share of conjugated linoleic acids (*cis-9*,*trans-11* C18:2) is synthesized in the ruminant tissues and particularly in the mammary gland by the desaturation of *trans*-vaccenic acid (*trans-11* C18:1) resulting from the action of the stearylo-CoA desaturase (SCD). The introduction of *cis*-double bond is catalysed by the set of microsomal electron-transport proteins composed sequentially of NADH cytochrome *b5* reductase, cytochrome *b5* and the terminal SCD. Stearylo-CoA desaturase is the rate-limiting component in this reaction. Its activity is affected by different factors such as diet, hormones, temperature, metals, peroxisomal proliferators, vitamin A, and developmental processes [Ntambi 1999, Miyazaki and Ntambi 2003]. It can be hypothesized that supplementation of lambs diet with Se, Zn and vitamin E can regulate the activity of SCD.

Enrichment of ram-lambs diet with vitamin E at a rate exceeding recommendations, significantly reduced lipid oxidation, drip loss and tended to maintain meat redness [Macit *et al*. 2003]. We suggest that dietary Se, Zn and vitamin E decreased the β-oxidation of the CLA isomers in muscles. Therefore, the results presented here are in accordance with other studies in which dosed selenate, as strong antioxidant, stimulated driving fatty acids along the oxidation pathway [Czauderna *et al*. 2003, 2004b]. It can be hypothesized that both Se (as Na_2SeO_4) and vitamin E have a protective effect against peroxidation damage in catabolism on CLA isomers.

This study demonstrated that Se, Zn and vitamin E administered orally to growing ram-lambs induced a decrease in cholesterol content of blood and meat, and led to increased the CLA isomer level in meat and liver. Based on the above observation, it is suggested that the diet enriched in Se, Zn and vitamin E improved the lipid profile of lamb's meat as assessed from the consumer's point of view.

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Wpływ Se, Zn i witaminy E na zawartość cholesterolu, CLA i innych kwasów tłuszczowych w mięsie jagniąt

S t r e s z c z e n i e

Badania przeprowadzono na 20 jagniętach merynosa polskiego, podzielonych na 2 grupy: doświadczalną (E) i kontrolną (C). Po odsadzeniu w wieku 8 tygodni, jagnięta umieszczono w klatkach indywidualnych. Jagnięta tuczono do średniej masy ciała 31,8 kg, karmiąc je do woli pełnoporcjową granulowaną mieszanką treściwą i sianem. Podczas tuczu codziennie podawano każdemu jagnięciu z grupy E *per os* następujące związki: 1 ml 0,1% Na_2SeO_4, 3 ml 10% $ZnSO_4$ i 60 mg witaminy E (α-tocopherol). Oznaczono zawartość cholesterolu, SFA, MUFA, PUFA i CLA w polędwicy i w wątrobie jagniąt, jak również cholesterol całkowity i frakcję HDL oraz trójglicerydy we krwi. Stwierdzono, że polędwica jagniąt, którym podawano Se, Zn i witaminę E, zawierała istotnie więcej CLA (0,45%), a mniej cholesterolu (42,8 mg/100 g świeżej masy) w porównaniu z grupą kontrolną. Zawartość CLA w wątrobie jagniąt doświadczalnych również była istotnie wyższa. Stężenie cholesterolu całkowitego we krwi jagniąt doświadczalnych obniżyło się istotnie, a frakcji HDL wzrosło. Wnioskuje się, że dodanie do dawki pokarmowej dodatkowej ilości Se, Zn i witaminy E poprawia profil lipidowy mięsa jagnięcego.