

The Influence Of Isomerised Plant Oils With High Content Of Conjugated Dienes Of Linoleic Acids *c9,t11* And *t10,c12* On The Fat Reduction In The Carcass And Meat of Lamb

¹Patkowska-Sokola B., ¹Bodkowski R., ²Walisiewicz-Niedbalska W. and ³Zielinski S.

¹Institute of Animal Breeding, Wrocław University of Environmental and Life Sciences, ul Chelmonskiego 38c, 51-630 Wrocław, Poland

²Institute of Industrial Chemistry Research im. I. Moscickiego ul. Rydygiera 8, 01-793 Warsaw, Poland

³Institute of Inorganic Technology and Mineral Fertilizers, Wrocław University of Technology, ul. Smoluchowskiego 25, 50-372 Wrocław, Poland

Abstract: An influence of isomerised poppy seed oil on lamb body fat and fat content in meat, and also fatty acids content was determined in the research. As a result of poppy seed oil isomerisation, the synthesis of conjugated dienes of linoleic acid of *trans-10, cis-12* and *cis-9, trans-11* configuration in amounts 34.3% and 32.2%, respectively, occurred. The enrichment of feed dose of fattening lambs with this additive caused body fat reduction at a level from 13 to 37%, and a lowering of fat content in muscular tissue from 13 to 27 percentage points. An addition of isomerised poppy seed oil also profitably modified the content of fatty acids in adipose tissues causing an increase of biologically active beneficial components such as: *c9,t11* and *t10,c12* isomers of linoleic acid, vaccenic acid, and a decrease of saturated fatty acids content.

Key words: lambs, isomerised poppy seed oil, CLA, body fat, fatty acids profile

INTRODUCTION

Research results so far indicate that some monounsaturated fatty acids with *trans* configuration may inversely influence lipid management increasing the risk of hyperlipidemia and the development of atherosclerosis^[1]. An unprofitable role is also attributed to *trans* isomers of polyunsaturated fatty acids. For example, linoleic acid of *t9,t12* configuration decreases the conversion of linoleic acid of *cis* configuration to arachidic acid, that in turn is a reason for prostaglandines synthesis decrease^[2]. In turn, *c9,t12* isomer via Δ^6 -desaturase inhibition decreases biosynthesis of long-chain polyunsaturated fatty acids and their transformation products (eicosanoids)^[3].

However, not all *trans* isomers of unsaturated fatty acids show unprofitable activity. A positive and multiple role is attributed, for example, to conjugated dienes of linoleic acid (CLA).

These compounds have been known for a long time, however until the mid 1980s not much was known about their chemical properties and activity. Only

research on heterocyclic aromatic amines, conducted by Pariza *et al*^[4], led to the unexpected discovery of compounds inhibiting mutagenesis in the *Salmonella typhimurium* bacteria in the fatty extracts of raw and fried beef meat. The presence of compounds inhibiting carcinogenesis, in chemically induced cancer, in beef meat was also confirmed in *in vivo* research on mice and rats^[5]. As it appeared, the compounds were just conjugated dienes of linoleic acid. Since that time, many other biological and physiological functions of these compounds have been recognised.

Conjugated linoleic acid (CLA) is a common term describing the mixture of positional (8 and 10, 9 and 11, 10 and 12 or 11 and 13), and geometric (*cis* and *trans*) isomers of octadecanoic acid C18:2, where, unlike in linoleic acid, double bonds are isolated by only one single bond (i.e. are conjugated)^[6]. In natural products isomers of *cis-9, trans-11* (*c9,t11*) configuration predominate and the particular biological activity is attributed to just these dienes^[7].

The main mechanism of conjugated dienes of linoleic acid (CLA) creation is an isomerisation of

Corresponding Author: Patkowska-Sokola B., Institute of Animal Breeding, Wrocław University of Environmental and Life Sciences, ul Chelmonskiego 38c, 51-630 Wrocław, Poland

linoleic acid that takes place during catalytic hydrogenation of plant oils. Created this way isomers are, however, insubstantial, and that is the reason for their low content in margarines, for example. These isomers are created also during thermal treatment of food. However, the biggest amounts of them are created as a result of bacterial biohydrogenation of linoleic acid in a rumen of ruminants (cows, sheep, goats). That is why products coming from this group of animals, i.e. milk and its preserves, and meat, are the most important source of these isomers in human diet.

Conjugated dienes of linoleic acid undergo changes to furane forms of antioxidant properties and to conjugated dienes of linolenic and eicoatrienic acid in human and animal organisms^[8]. Probably, due to the strong antioxidative properties of CLA and their metabolites, they have a profitable impact on human and animal organisms^[9]. Moreover, the conjugated diene of *cis*-9, *trans*-11 configuration may be incorporated in membrane structures of cells modifying their properties (liquidity, permeability).

Thus far most research has been devoted to the anticarcinogenic activity of conjugated dienes of linoleic acid. In *in vitro* research on human cancer cells and *in vivo* on mice and rats, it has been demonstrated that conjugated dienes of linoleic acid, mainly *cis*-9, *trans*-11 isomer, delay or decrease the development of skin, breast, colon and stomach cancer^[10, 11, 12]. Also clinical research on humans, however to a less extent, confirm the antimutagenic and anticarcinogenic properties of CLA^[13, 14].

The profitable spectrum of conjugated dienes of linoleic acid activity, especially *cis*-9, *trans*-11 isomer, is however not limited only to mutagenesis and carcinogenesis inhibition, but, as it is stated in the literature, is much wider and multiple. Conjugated dienes of linoleic acid also show hypercholesterolemic and anti-atherosclerosis^[15, 16], inhibiting osteoporosis development^[17], immunomodulating^[18], and diabetes development deleting^[19] activity.

Conjugated dienes of linoleic acid, especially isomer of *trans*-10, *cis*-12 configuration, may also improve fodder intake and carcass mass gains at the cost of decrease of fat content in body mass^[20, 21].

Currently, one of the more important problems in breeding is a decrease of animal body fat. In the face of this expectation, the authors of the present paper have conducted research on the possibility of the use of

isomerised poppy seed oil enriched via synthesis with conjugated dienes of linoleic acid of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 configuration in lamb fattening.

MATERIALS AND METHODS

Gaining and determining the fatty acids content of poppy seed oil: Poppy seed oil was cold pressed from low-morphine poppy seed strain – „Michalko”. Fatty acids content was determined using the capillary gas chromatography method on Hewlett-Packard II apparatus with flame-ionization detector (FID) and 50m long capillary column CP Sil 88.

Separation conditions. Temperature of column - 170°C, dosimeter - 200°C, detector - 250°C, carrier gas – helium.

Methyl esters were obtained according to AOCS Official Methods Ce2-66. Analyses were carried out according to AOCS Official Methods Ce 1f-96. Quality identification was done by the comparison of retention times of analysed components with models. Heptadecanoic acid C17 was used as an internal standard.

Syntheses of conjugated dienes of linoleic acid *cis*-9,*trans*-11 and *trans*-10,*cis*-12 from *cis*-9, *trans*-12 C18:2 acid.

Initial substrate: Low-morphine poppy seed oil.

Conditions of isomerisation: temperature 180°C, pressure – normal; reaction environment – distilled glycerine of 99% purity, molar ratio of reagents: 1 mole of oil + 60 moles of glycerine + 7,4 moles of NaOH; time of the reaction – 3 hours.

Method of conducting the reaction. Glycerine was placed in a reactor, warmed to a temperature of 50-60°C and NaOH was introduced. The whole mixture continued to be warmed to a temperature of about 140°C, keeping mixing, until hydroxide dissolution. Next, the oil was introduced, and the temperature of reaction was raised to 180°C. The process was carried out for 2-3 hours in such conditions. After the end of reaction and cooling the reactor content to below 100°C, water was introduced in an amount of 1:1 (v/v) in order to dilute arisen soap solution. Next, sulphuric acid was introduced to the reactor in order to acidify soaps to the form of free fatty acids. The process was conducted at a temperature of 70-80°C for half an hour.

After the complete acidification of soaps, the reaction mixture was placed in a separator, where water phase was separated, and a phase of fatty acids was washed with hot water (about 90°C) to the neutral pH of washing water. After the complete washing, acids were dried above sodium sulphate. Such prepared product was analyzed.

Analytical methods: Qualitative identification was done by the comparison of retention times of analysed components with standards. As standards CLA isomers by Sigma Company were used. The identification of positional isomers of the main components was conducted by GC/MS method, using the specific fragmentation of fatty derivatives with 2-amino-2-methyl-1-propanol (DMOX). The separation of methyl esters of trans and cis fatty acids was done using thin-film chromatography TLC-Ag⁺. The collected bands of methyl esters corresponding to trans and cis fractions were transformed to their derivatives with 4,4-dimethyloxazoline. Obtained DMOX derivatives were analyzed using GC/MS method.

The use of isomerised poppy seed oil in lambs feeding: In the second part of the research, isomerised poppy seed oil, enriched by isomerisation in conjugated dienes of linoleic acid *t10,c12* and *c9,t11*, was placed via the nozzle shower method on a humic-mineral carrier - „Humokarbawit”. The amount of placed oil was 18% per 1kg of carrier. The animal research material consisted of 60 randomly chosen ram lambs aged about 3 months: 30 Friesian bred, body mass about 18±2.0 kg and 30 Longwool bred, body mass 22±1.8 kg. All animals were fed indoors. The feeding dose was established according to current standards for fattening lambs, based on CJ mixture and meadow hay. Animals had constant access to water and licks. According to methodological assumptions, 4 groups were created, 15 heads in each (2 control and 2 experimental). The experiment lasted 6 weeks. During the whole experiment lambs from experimental groups were given a daily supplement of Humokarbawit with isomerised poppy seed oil in amount 50g/head/day, while lambs from control groups were given Humokarbawit itself in the same amount. The amount of supplement in experimental groups was established so that the effective daily dose of trans-10, cis-12 isomer was 3g/head. After the end of the experiment all lambs were slaughtered and perinefric fat from the area of kidney mesentery and subcutaneous and intermuscular fat from the leg, and also longissimus

dorsi muscle (*musculus longissimus dorsi*) and quadriceps muscle of the leg (*musculus quadriceps femoris*). Perinefric, subcutaneous and intermuscular fat was weighed and its fatty acids profile was determined. In turn, from the longissimus dorsi muscle and quadriceps muscle of the leg, the intramuscular fat was extracted and its amount and fatty acids content were determined.

Fat extraction from muscular tissues was done according to Folch method (chloroform + methanol in a ratio 2:1). Fatty acids content was determined using capillary gas chromatography method on PU 4410 apparatus (Philips) with flame-ionization detector (FID) and 105m long capillary column Rtx-2330.

Conditions of separation. Initial isotherm– 160°C (30 min) – 3°C/min. to 180°C – 17 min. in temperature of 180°C, for 5 min. to 210°C – 20 min. in temperature of 210°C. Other conditions: temperature of column – 160°C, temperature of detector– 230°C, temperature of a chamber – 220°C, carrier gas – Helium 80 PSI.

Qualitative identification was conducted by comparison of retention times of obtained peaks with retention times of Sigma Company standards.

Isomerisation of poppy seed oil and the determination of fatty acids content was done at the Institute of Industrial Chemistry Research in Warsaw, Poland.

To assess the significance of differences between analysed parameters SAS (1996) statistical software was used.

RESULTS

Poppy seed oil was characterised by a high content of linoleic acid C18:2, about 73.1%, and by a low content of linolenic acid C18:3, about 0.5%, and saturated acids C14-18, about 12% (Table 1). Similarly like other unprocessed oils of plant origin, it did not include conjugated dienes of linoleic acid (CLA). As a result of the process of its isomerisation, there was a synthesis from linoleic acid of dienes of linoleic acid of *cis-9, trans-11* and *trans-10, cis-12* configuration in amounts, respectively, 32.2 and 34.3% of all fatty acids pool (Table 1). Apart from CLA, the isomerised poppy seed oil included also saturated fatty acids of C16-18 chain length - 11.3%, acids: oleic, linoleic and linolenic in amounts 14.9, 6.6 and 0.3%, respectively, and isomers of linolenic acid in amount <0.1% (Table 1).

Pictures 1-4 present the results concerning the influence of isomerised poppy seed oil addition on body fat of lambs and fat content in their meat.

Table 1: The composition of fatty acids of poppy seed oil before and after the isomerisation (%)

Fatty acids	percentage participation of fatty acids in poppy seed oil	
	before isomerisation	after isomerisation
C14:0	trace	trace
C16:0	10.2	9.2
C16:1	0.1	< 0.1
C18:0	1.8	2.1
C18:1	14.2	14.9
C18:2	73.1	6.6
C18:2 <i>c9,t11</i> isomer	-	32.2
C18:2 <i>t10,c12</i> isomer	-	34.3
C18:3	0.5	0.3
C18:3 isomers	-	< 0.1
C20:1	trace	-

In case of Friesian lambs, the supplementation with isomerised poppy seed oil caused a decrease of perinefric fat content of about 37% ($P \leq 0.01$) and subcutaneous and intermuscular fat content in leg of about 17% ($P \leq 0.05$) and 13%, respectively (Fig. 1).

Also the intramuscular fat content in longissimus dorsi muscle decreased by about 21 percentage points ($P \leq 0.01$) and in the quadriceps muscle of the leg by about 14 percentage points ($P \leq 0.05$) (Fig. 2).

In turn, in the case of Longwool lambs, the amount of perinefric fat in the carcass decreased by about 33% ($P \leq 0.01$), and the amount of subcutaneous and intermuscular fat in by about 15% and 17% ($P \leq 0.05$), respectively (Fig. 3).

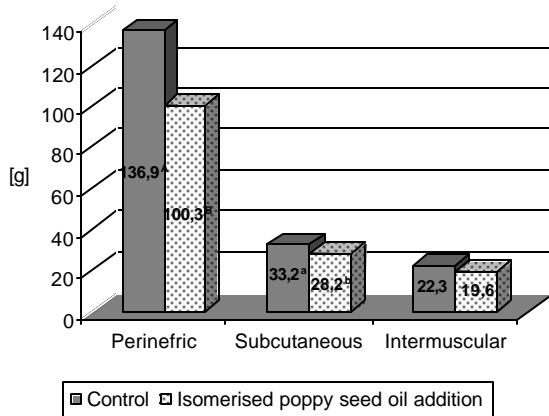


Fig. 1: The content of perinefric fat in carcass and subcutaneous and intermuscular fat in leg of Friesian lambs (g).

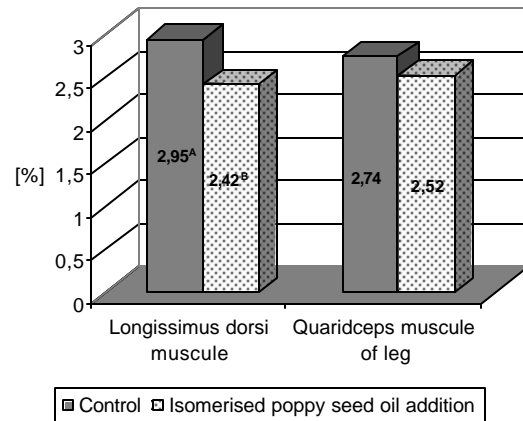


Fig. 2: The content of intramuscular fat in longissimus dorsi muscle (*musculus longissimus dorsi*) and in quadriceps muscle of leg (*musculus qadriiceps femoris*) of Friesian lambs (%).

Similar to the case of Friesian lambs, also in this group there was a decrease in intramuscular fat content in longissimus dorsi muscle and in quadriceps muscle of the leg of about 28 percentage points ($P \leq 0.01$) and 17 percentage points ($P \leq 0.05$), respectively (Fig. 4).

Tables 2 and 3 present the fatty acids profile of adipose tissues. For the reason of frame restrictions, only results concerning the fatty acids content of intermuscular and intramuscular fat are presented here, as it is these that are of the most importance to the consumer since they cannot be removed during culinary treatment.

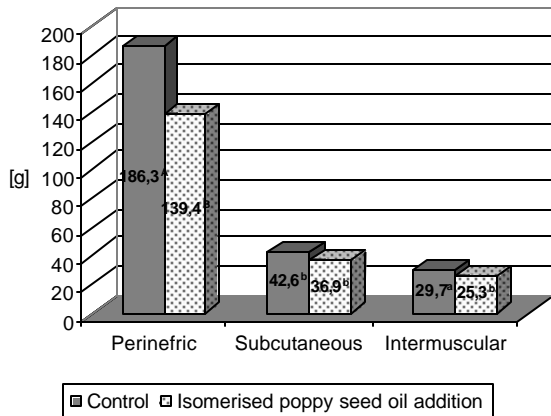


Fig. 3: The content of perinefric fat in carcass and subcutaneous and intermuscular fat in leg of Longwool lambs (g).

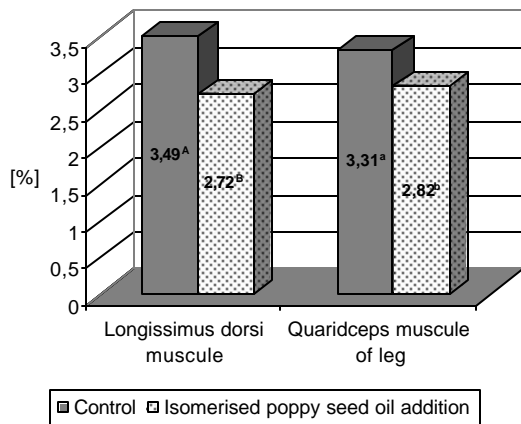


Fig. 4: The content of intramuscular fat in longissimus dorsi muscle (*musculus longissimus dorsi*) and in quadriceps muscle of leg (*musculus qadriceps femoris*) of Longwool lambs (%).

An addition of isomerised poppy seed oil to the feeding dose of fattening lambs caused a slight decrease in saturated fatty acids content and an increase in

monounsaturated and polyunsaturated fatty acids content in intermuscular fat (Table 2). Differences, however, were not statistically significant. Significant differences were observed in the content of particular fatty acids. In both experimental groups there was a decrease in the content of short-chain saturated fatty acids (C8-13:0): of 28.5 percentage points ($P \leq 0.05$) in the case of Friesian lambs, and of 45.1 percentage points ($P \leq 0.01$) in the case of Longwool lambs (Table 2). There was also a decrease in miristic acid (C14:0) content of 15.6 and 20.5 percentage points ($P \leq 0.05$) respectively, and in case of Friesian lambs also the decrease in pentadecanoic acid (C15:0) content of 15.6 percentage points ($P \leq 0.05$), and saturated fatty acids of C20-24 chain length of 21.5 percentage points ($P \leq 0.05$) (Table 2). However, an increase in stearic acid (C18:0) content was observed: 6.2 percentage points in case of Friesian lambs ($P \leq 0.05$), and 9.1 percentage points for Longwool lambs ($P \leq 0.01$) (Table 2).

In a group of monounsaturated fatty acids, the biggest changes were noted in a range of an isomer of oleic acid – vaccenic acid t18:1n7v content. The participation of this acid in Friesian lambs increased by 119.5 percentage points ($P \leq 0.01$), while in case of Longwool lambs by 127.7 percentage points ($P \leq 0.01$) (Table 2). There was however a decrease in palmitooleic acid (C16:1) content of 24.2 percentage points ($P \leq 0.05$), and 31.5 percentage points ($P \leq 0.01$), respectively (Table 2).

In turn, in a group of polyunsaturated fatty acids the most significant changes were noted for the content of conjugated dienes of linoleic acid. As a result of isomerised poppy seed oil addition, the content of conjugated diene of linoleic acid of cis-9, trans-11 configuration increased in Friesian and Longwool lambs fat of 51.8 and 96.7 percentage points ($P \leq 0.01$) respectively, while the content of isomer of trans-10, cis-12 configuration of 52.2 and 60.7 percentage points, respectively ($P \leq 0.01$) (Table 2).

Table 2: Fatty acids content in intermuscular fat of Friesian and Longwool lambs (%)

Fatty acids	Friesian		Longwool	
	Control	Experimental*	Control	Experimental*
C8-13:0	1.240 ^a	0.887 ^b	1.534 ^A	0.842 ^B
C14:0	3.675 ^a	3.102 ^b	4.881 ^a	3.882 ^b
C15:0	1.721 ^a	1.452 ^b	1.634	1.542
C16:0	18.243	16.102	18.650	16.213
C17:0	3.421	3.134	3.284	2.943
C18:0	30.234 ^a	32.102 ^b	31.467 ^A	34.325 ^B
C20-24:0	0.567 ^a	0.445 ^b	0.466	0.427

Σ Saturated	59.101	57.224	62.916	60.674
C14:1	0.633	0.582	0.538	0.496
C16:1	2.654 ^a	2.011 ^b	2.124 ^A	1.456 ^B
C18:1	28.535	30.042	26.267	27.123
tC18:1n9e	0.156	0.142	0.122	0.103
tC18:1n7v	1.123 ^A	2.465 ^B	1.176 ^A	2.678 ^B
Σ Monounsaturated	33.501	35.242	31.217	31.856
C18:2	3.405	3.726	2.611	3.342
C18:2 c9,t11	0.304 ^A	0.665 ^A	0.342 ^A	0.673 ^B
C18:2 t10,c12	0.067 ^B	0.102 ^B	0.107 ^A	0.132 ^B
other C18:2 isomers	0.028	0.023	0.048	0.056
C18:3	1.821	2.011	1.456	1.250
C20:2 – 20:5	0.756	0.565	0.876	0.767
C22:4 - 22:6	0.286	0.242	0.352	0.332
Σ Polyunsaturated	6.767	7.334	5.792	6.552

* -isomerised poppy seed oil addition

^{A,B} – differences significant on the level $P \leq 0.01$

^{a,b} – differences significant on the level $P \leq 0.05$

Similar tendencies to those identified in intermuscular fat were also observed in intramuscular fat. As a result of isomerised poppy seed oil addition, the content of saturated fatty acids was slightly decreased, while the content of monounsaturated and polyunsaturated acids was increased (Table 3). In a group of saturated fatty acids the decrease applied mainly to the content of short-chain saturated fatty (C8-13:0) and was 21.2 percentage points in the case of Friesian lambs ($P \leq 0.05$), and 25.1 percentage points in the case of Longwool lambs ($P \leq 0.01$). There was also a decrease in miristic acid (C14:0) content of, respectively, 22.7 and 10.1 percentage points ($P \leq 0.05$), and palmitic acid (C16:0) respectively of 14.1 and 13.9 percentage points ($P \leq 0.05$) (Table 3). However, an increase in stearic acid (C18:0) content of, respectively, 12.9 and 10.6 percentage points ($P \leq 0.05$) was observed (Table 3).

In a group of monounsaturated fatty acids, a statistically significant increase, as a result of a supplementation with isomerised poppy seed oil, was noted only in case of vaccenic acid content. The participation of this acid increased respectively by 105.8 and 85.8 percentage points ($P \leq 0.01$) for Friesian and Longwool lambs from experimental groups (Table 3).

In turn, in a group of polyunsaturated fatty acids for lambs receiving an addition of isomerised poppy seed oil, there was an increase in conjugated dienes of linoleic acid content. In the case of Friesian and Longwool lambs, the increase was respectively 148.1 and 56.6 percentage points ($P \leq 0.01$) for the isomer of *cis-9, trans-11* configuration, and 73.2 and 68 percentage points ($P \leq 0.01$) for the isomer of *trans-10, cis-12* configuration (Table 3).

Table 3: Fatty acids content in intramuscular fat of longissimus dorsi muscle (*musculus longissimus dorsi*) in Friesian and Longwool lambs (%)

Fatty acids	Friesian		Longwool	
	Control	Experimental*	Control	Experimental*
C8:0 – 13:0	1.431 ^a	1.128 ^b	1.212 ^A	0.908 ^B
C14:0	2.885 ^a	2.231 ^b	3.652 ^a	3.282 ^b
C15:0	2.371	2.304	1.589	1.492
C16:0	22.243 ^a	19.102 ^b	23.232 ^a	20.011 ^b
C17:0	3.941	3.902	2.971	2.656
C18:0	22.234 ^a	25.102 ^b	25.327 ^a	28.025 ^a
C20:0 – 20:4	0.736	0.685	0.472	0.458
S Saturated	55.841	54.454	58.455	56.832

C14:1	0.435	0.516	0.327	0.311
C16:1	2.123	1.832	2.924	2.299
C18:1	34.435	35.042	32.176	32.967
tC18:1n9e	0.121	0.109	0.082	0.089
tC18:1n7v	1.318 ^A	2.712 ^B	1.021 ^A	1.897 ^B
S Monounsaturated	38.432	40.211	36.530	37.563
C18:2	3.147	2.726	2.312	2.695
C18:2 c9,t11	0.386 ^A	0.572 ^B	0.401 ^A	0.628 ^B
C18:2 t10,c12	0.056 ^A	0.097 ^B	0.097 ^A	0.163 ^B
other C18:2 isomers	0.016	0.014	0.011	0.009
C18:3	1.214	1.236	1.287	1.250
C20:2 – 20:5	0.536	0.425	0.411	0.367
C22:4 - 22:6	0.207	0.237	0.202	0.176
S Polyunsaturated	5.562	5.307	4.721	5.288

* -isomerised poppy seed oil addition

^{A,B} – differences significant on the level $P \leq 0.01$

^{a,b} – differences significant on the level $P \leq 0.05$

DISCUSSION

The fatty acids profile of initial substrate has a significant impact on the amount of synthesised by isomerisation conjugated dienes of linoleic acid *trans-10,cis-12* and *cis-9,trans-11*. The high content of C18:2 *cis-9,trans-12* linoleic acid that is the main substrate in a process of these isomers synthesis, and the low content of C18:3 *cis-9,cis-12,cis-15* linoleic acid that also undergo isomerisation creating positional and geometric isomers of unknown biological properties are main determinants of its high usefulness. Also, the low content of saturated fatty acids is profitable for the synthesis of conjugated dienes of linoleic acid, however they can be removed from the final product by the crystallization from urea and extraction in supercritical CO₂ conditions, increasing, in this way, the concentration of desirable isomers in acids pool^[22].

The results of chromatographical analysis showed that poppy seed oil is characterised by profitable, for the possibility of the synthesis of conjugated dienes of linoleic acid (CLA), content of fatty acids, since it contains a high amount of linoleic acid (C18:2) and a relatively low amount of linolenic acid (C18:3) and saturated acids. A similar profile of fatty acids is observed in sunflower seed and Amaranthus oils^[23].

The isomerisation process that poppy seed oil was subjected to, caused the changes in the placement of double bonds in chains of unsaturated fatty acids (positional) and the changes of radicals arrangement in relation to the axis of double bond (geometric). As a result, two, not occurring naturally in poppy seed oil, conjugated dienes of linoleic acid of *cis-9, trans-11* and *trans-10, cis-12* configuration and of properties quite different from C18:2 c9,c12 linoleic acid that they originate from, were created.

Since isomerised poppy seed oil had an oleic form, it was mixed with a carrier to make it easier to give it to lambs. In the present study, the humic-mineral preparation „Humokarbovit” that is suitable in different animal species feeding, for example as a supplement with biostimulating properties^[24], was used. It contains natural humic-mineral substances including humic acids and their salts, bitumines, hemicellulose, lignin, wax, resins, phytohormones, phytoenzymes, proteins and aminoacids, polysugars and a rich combination of macro- and microelements. The preparation is characterised by high sorptive ability and antioxidant properties.

Conducted research with the use of isomerised poppy seed oil supplement of high content of t10,c12 and c9,t11 linoleic acid isomers in lambs feeding dose, proved its profitable effect on the decrease in animal body fat, that depending on the kind of adipose tissue was 13 to 37%, and on lowering of fat content in their muscular tissue from 13 to 27 percentage points.

The profitable effect of CLA supplementation on animal body fat reduction is also confirmed by results of other authors' work. An addition of 0.5% of CLA to mixtures for mice, rats and chickens caused the reduction of adipose tissue of 55, 23 and 22%, respectively, with the increase in muscular tissue participation of 5-14, 3 and 4%, respectively, at the same time^[25]. Enriching the mice fodder with the same amount of CLA, Park *et al*^[26] obtained the reduction of adipose tissue of about 60% after 4 weeks of experiment. Also Steinhart^[27] observed decreased body fat, faster growth rate, and better fodder utilisation in cases of young rats receiving an addition of this acid. In cases of swine, the reduction of body fat was 27%, and the increase in lean body mass participation of about

5%. An addition of 0.5% CLA for three months was used in this research^[28].

An influence of conjugated dienes of linoleic acid on body fat reduction was also confirmed in other research on mice^[29, 30, 31, 32], rats^[33], swine^[34, 35, 36] and humans^[37].

It is known presently that the effects of CLA isomers influence on adipogenesis and fat metabolism in animals are dependent on the kind of isomer, dose, time of use and on the species of experimental animals. Especially trans-10, cis-12 isomer seems to be responsible for decreasing of body fat in animals and obesity in human, since it has an effect on preadipocytes^[21, 38]. This isomer decreases the triglyceride content in differentiating preadipocytes, and this effect is reversible and dependent on the time of use^[39, 40]. Trans-10, cis-12 isomer also decreases *de novo* synthesis of fatty acids and the esterification of triglycerides in human preadipocytes. C18:2 isomers (cis-9, trans-11 vs trans-10, cis-12) have an opposite effect on lipids metabolism. Isomer c9,t11 improves lipids metabolism, while t10,c12 contributes to the decrease of adipose tissue.

Trans fatty acids, including isomers of C18:2 acid, act as modulators of lipid metabolism^[41]. In a group of obese and also overweight people, the addition of 3.4g CLA daily statistically significantly reduced body mass^[42]. In turn, Griinardi *et al*^[43] conducted research indicating that with the lowered fat synthesis in milk from dairy cows there was an increase in trans-10, cis-12 C18:2 isomer content. The synthesis of milk fat was also lowered by trans-10, cis-12 isomer occurring in a fodder^[44].

The mechanism of conjugated linoleic acid (CLA) activity as a factor reducing body fat is not fully recognized. Probably it proceeds two-way. First, CLA inhibits lipoprotein lipases activity, that are placed in blood vessels lining of muscles and other tissues, using fat as an energy source. The lipases are responsible, among others, for the hydrolysis of very low density lipoproteins (VLDL).

Secondly, conjugated linoleic acid increases activity of lipases placed directly in adipocytes. Via hormonal regulation (adrenaline, noradrenaline and adrenocorticotropin) there is a stimulation of adenylyl cyclase and protein kinase, and lipases phosphorylation as a result. Induced this way, lipolysis is the first step towards the utilization by an organism of emergency fat, not dietary fat, as an energy source.

The ability of CLA to modify relative participation of adipose tissue is also explained by their thermogenic properties.

Besides the decrease in body fat and the reduction of fat content in muscular tissue, an addition of

isomerised poppy seed oil was found to modify profitably the fatty acids profile. There was a significant increase in biologically active components of beneficial for the health activity like conjugated dienes of linoleic acid c9,t11 and t10,c12, and vaccenic acid, and there was a decline in the content of acids with attributed atherogenic activity i.e. short-chains saturated fatty acids and palmitic acid.

Observed differences in fatty acids content between adipose tissues of lambs receiving an addition of isomerised poppy seed oil, and lambs that were not given the addition, are probably due to the different supply of conjugated dienes of linoleic acid (CLA) in their dietary doses, that undergo further changes in rumen of ruminants. An unquestionable majority of these isomers, with the participation of rumen microorganisms, are subjected to biohydrogenation, first to trans vaccenic acid C18:2 n7v and then to stearic acid, and in this form it incorporated into adipose tissues. However, a part of conjugated dienes of linoleic acid in unchanged form is transferred to further parts of alimentary canal, where it is absorbed and then incorporated into adipose tissues. Moreover, vaccenic acid is also used in adipose tissues as a substrate for endogenous CLA synthesis with the ?⁹-desaturase participation.

The ability, observed in the present study, of the conjugated dienes of linoleic acid to reduce body fat and fat enrichment in biologically active compounds of beneficial for health properties is a valuable observation.

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