Animal Science Papers and Reports vol. 31 (2013) no. 3, 229-238
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Reduction of body fatness and meat fat content in lambs by supplementing their diet with isomerised grapeseed oil*

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(Received September 18, 2012; accepted July 4, 2013)

Investigated was the effect of isomerised grapeseed oil supplementation on carcass composition and body fat in lambs and fat content of their meat. Grapeseed oil alkaline isomerisation and crystallisation with urea resulted in synthesis of conjugated dienes amounting to linoleic acid (CLA) 77.6% FFA. The enrichment of fattening lambs´ rations with the additive at 18 g/animal/day did not influence body weight gain, carcass weight and *Longissimus dorsi* parameters (weight, width, depth). However, it reduced body fatness by 19 to 25%, and decreased the fat content of muscle tissue by 17 to 22 percentage points.

KEY WORDS: body fat / carcass composition / dietary CLA / lambs / meat fat

Currently, one of more important problems is the overly high consumption of saturated fatty acids, the main source of which in human diet are animal fats. According to doctors and dieteticians these compounds are associated with increased lipid parameters of blood (triglycerides, total cholesterol and LDL), which lead to arterosclerosis and contributes to cardiovascular diseases [Siri-Tarino *et al*. 2010] Saturated fatty acids are also responsible for some types of cancer [Rose 1997] and obesity [Bray *et al*. 2002]. The consumption of saturated fatty acids should therefore

*Carried out as a part of Research Project 3 T09B 130 29 financed by the Polish Ministry of Science and Higher Education. The developed feed supplement was patented in the Patent Office of the Republic of Poland - PL 386645
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be limited, and one way of doing so is to reduce fatness of animals and fat content of their meat, inter alia by suplemmenting animal's diet with plant oils enriched with n-3 fatty acids characterising health promoting properties [Poławska *et al*. 2011, 2013].

Sheep fat is also characterised by an unfavourable fatty acids composition, which, depending on the type of fatty tissue and feeding, contains from 55 to 68% of saturated fatty acids [Jamroz *et al*. 2002]. Decreasing the fat:lean ratio and altering the composition of sheep tissues may be beneficial for improving the nutritional quality of lamb meat.

Park *et al*. [1997] were the first to report that dietary CLA could decrease body fat mass and increase lean body mass. This positive effect of CLA supplementation on body fat reduction in several animal models (rodents, pigs, poultry) is also confirmed by the results of other authors [Azain *et al*. 2000*,* DeLany *et al*. 1999, Du and Ahn 2002, Dugan *et al*. 1997*,* Ostrowska *et al*. 1999*,* Tsuboyama-Kasaoka *et al*. 2000, West *et al*. 1998].

The aim of this study was the supplementation of the diet of lambs with CLA (synthesized from grapeseed oil) and the determination of that supplement's influence on carcass composition and adiposity rate.

Material and methods

Enrichment of grapeseed oil with CLA

Grapeseed oil was enriched with CLA following the method of Walisiewicz-Niedbalska *et al*. [2009], at the Industrial Chemistry Research Institute in Warsaw, Poland. Fatty acids profile of grapeseed oil before and after alkaline isomerisation and crystallisation with urea is presented in Table 1.

Preparation of feed additive

Due to its oily form, isomerised grapeseed oil (IGO) was applied to a mineral carrier to make it more applicable to lamb feeding. We used a mineral humic preparation (*Humokarbowit*). For this purpose IGO was applied at 18% per kg of the carrier using a nozzle spray.

Animals and diets

Subjects were 40 randomly chosen Polish Merino ram lambs aged about 8 weeks and weighing 22±1.2 kg, divided into two equal groups (control and experimental) with 20 animals per group. All lambs were fed indoors. The ration was formulated according to the INRA system [IZ-PIB-INRA, 2009] using current standards for fattening lambs based on concentrate feed and grassland hay (Tab. 2). Additionally, throughout the experiment, lambs from the experimental group were supplemented with *Humokarbowit* and isomerised grapeseed oil (IGO) at 100 g/head/day, while control lambs received the same amount of *Humokarbowit* alone (Tab. 2). All the

Table 1. Content of main fatty acids (%) in grapeseed oil and in the product obtained after its alkaline isomerisation and crystallisation with urea (IGO)

Fatty acid	Grapeseed oil (%)	
	before	after isomerisation and crystallisation with urea (IGO)
C14:0	1.2	0.2
C16:0	7.8	0.3
C18:0	3.8	0.2
c9 C18:1	17.4	19.8
c9c12 C18:2	68.6	1.6
c9.t11 C18:2 (CLA isomer)	-	38.3
t10.c12 C18:2 (CLA isomer)	-	35.6
c11.t13 C18:2 (CLA isomer)	-	3.7
c9c12c15 C18:3	0.5	0.1
isomers C18:3	-	0.2
SFA	13.5	0.7
UFA	86.5	99.3
including		
MUFA	17.5	19.8
PUFA	69.0	79.5
CLA	-	77.6

SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.
SFA – Σ C12:0. C14:0. C15:0. C16:0. C18:0; MUFA – C18:1 *c9;* PUFA – Σ C18:2 *c9c12.* C18:2 *c9t11.* C18:2 *t10c12.* C18:2*11c.13t.* C18:3 *c9c12c15. C18:3 isomers*; UFA – MUFA + PUFA; CLA – Σ C18:2 *c9t11* + C18:2 *t10c12* + C18:2 *c11t13*.

animals had *ad libitum* access to water throughout. After 6 weeks of the experiment, all lambs were slaughtered at about 32±3.5 kg of body weight.

Slaughter, carcass and sampling procedures

At the end of the experiment all the lambs were slaughtered by exsanguination (*arteria carotis externa* cutting) following stunning (bolt pistol).

Immediately post-slaughter the carcasses were eviscerated, skinned and refrigerated at 6°C. Forty eight hours later the cold carcass weight was recorded before split in halves down the spinal column.

The following measurements were taken from the anterior surface of the left cross section: weight of the *M. longissimus dorsi* (LD), width of the LD (maximum distance across the cross-section of the muscle from the end adjacent to the spinal process, distal along the rib), depth of the LD (longest distance, perpendicular to width measurement, on the same surface), and thickness of subcutaneous fat over LD at the 3rd lumbar vertebra using a digital calliper.

Table 2. Composition (g/100 g) and nutritive value of concentrate mixture (UFV, PDIE, PDIN) for lambs daily intake (g/lamb) and daily intake of UFV, PDIE, PDIN

Item	Group	
	control	experimental
Compound of concentrate mixture (g/100g)		
wheat meal	55	55
barley grain	25	25
grassland hay	9	9
rapeseed meal	10	10
Polfamix OK*	1	1
Nutritive value of 1 kg concentrate mixture		
UFV	0.85	0.85
PDIE (g)	89.79	89.79
PDIN (g)	92.73	92.73
Daily intake (g/lamb)		
concentrate mixture	950	950
grassland hay	250	250
*Humokarbowit***	100	87
isomerised grapeseed oil****	-	18
Daily intake		
UFV	0.98	1.02
PDIE (g)	103.55	103.55
PDIN (g)	101.78	101.78

UVF – meat production unit; PDIE – protein digested in the small intestine when rumen-fermentable energy is limiting; PDIN – protein digested in the small intestine when rumen-fermentable nitrogen is limiting.
*1 kg of minerals (Polfamix OK) contains: 300 000 j.m. of vitamin A, 30 000 j.m. vitamin D3, 1.5 g of vitamin E, 0.5 g Fe, 2.5 g Zn, 65 g Mg, 0.015 g Co, 3 g Mn, 0.01 g J, 0.003 g Se, 60 g Na, 240 g C,. 120 g P.
***Humokarbowit* – including humic acids and their salts, bitumens, hemicellulose, lignin, wax, resins, phytohormones, phytoenzymes, proteins and amino acids, polysacharides and a wide range of macro- and microelements.
****Isomerised grapeseed oil (IGO) – contains CLA isomers: 38.3% *cis*9,*trans*11. 35.6% *trans*10,*cis*12. 3.7% *cis*11,*trans*13.

Within the experiment a dissection of the left leg was performed, with quantitative determination of the content of subcutaneous and intermuscular fat, and collecting of *M. longissimus dorsi* (LD) and *M. semitendinosus* (MS).

Measurement of the thickness of subcutaneous fat over the loin eye, weight, width and depth of LD, determination of the amount of subcutaneous and intermuscular fat of leg and extraction of intramuscular fat from LD and MS muscle tissues (according to a modified version of the method described by Folch *et al*. [1957]) were done at the Laboratory for Meat and Milk Analysis of the Wroclaw University of Environmental and Life Sciences, Poland.

Statistical

The effect of supplementing grapeseed oil enriched with CLA on the carcass characteristics, rate of fatness in lambs, fat content of their meat performed *via* one-way analysis of variance (ANOVA). A probability of $P \leq 0.05$ and $P \leq 0.01$ was adopted

as the criterion for significant differences. A STATISTICA 8.0 for Windows (StatSoft, Poland) software package was used. Differences among treatment means (groups) were verified for significance with Duncan test.

Results and discussion

The effect of CLA on body weight has been investigated in various animal models, including mice, pigs, steers and sheep [DeLany *et al*. 1999, Gassman 2000, Ostrowska *et al*. 1999, Park *et al*. 1997, Tsuboyama-Kasaoka *et al*. 2000, Wynn *et al*. 2006]. Most studies have shown that CLA decreases weight gain [Park *et al*. 1999, Terpstra *et al*. 2002, West *et al*. 1998], whereas others have shown no such effects [Ostrowska *et al*. 1999, Park *et al*. 1997, Wynn *et al*. 2006].

In this study no differences in growth rate between animals from control (227 g/day) and experimental (236 g/day) groups were found (Tab. 3). In addition, there were no effects of dietary isomerised grapeseed oil with CLA supplementation on any of the carcass traits composition. There were no differences in carcass weight and the weights, depth and width of LD between lambs fed different diets (Tab. 3).

Table 3. Growth. carcass characteristics and body fatness and meat fat content of lambs (mean±SD)

Item	Group	
	control*	experimental**
Gain (g/day)	227±18	236±24
Carcass wt (kg)	14.6±1.3	14.9±1.5
Backfat[1] (mm)	3.76^{A}±0.11	2.84^{B}±0.09
Subcutaneous fat wt[2] (g)	178.5^{a}±4.56	144.3^{b}±3.17
Intermuscular fat wt[3] (g)	82.1^{A}±3.77	62.8^{B}±4.13
Longissimus dorsi muscle		
weight (g)	510±35	498±27
width[4] (mm)	52.11±6.3	51.17±4.8
depth[5] (mm)	27.11±3.12	26.88±3.42
Intramuscular fat in *Longissimus dorsi* – MLD (%)	3.96^{A}±0.23	3.08^{B}±0.17
Intramuscular fat in *Semitendinosus* muscles – MS (%)	3.21^{a}±0.17	2.66^{b}±0.21

Control – standard concentrate mixture + grassland hay + *Humokarbowit.*
Experimental – standard concentrate mixture + grassland hay + *Humokarbowit* with isomerised grapeseed oil.
[1]Thickness of subcutaneous fat over *Longissimus dorsi* muscle.
[2]Content of subcutaneous fat in leg.
[3]Content of intermuscular fat in leg.
[4]Maximum distance across this MLD cross-section from the end adjacent to the spinal process, outwards along the rib.
[5]Longest distance, perpendicular to MLD width on the same surface.
[aA...]Means in rows marked with different superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

The observed lack of differences in body weight gain and carcass composition between animals from experimental and control group resulted probably from an insufficient level of dietary CLA (<0.2%) in the diet. It may, moreover, be related to

the application of dietary CLA in unprotected form, and as may be concluded from the study by Wynn *et al*. [2006], as much as 91.5% of CLA supplemented in a free acid form was subject to biohydrogenation in the rumen of sheep, whereas only 35% of the CLA was supplemented as a salt.

Similar observations in the range of lack of influence on any of the carcass component were noted by Wynn *et al*. [2006], who used an addition of protected CLA for sheep ranging from 25 to 100 g/kg of diet DM. No differences were found in growth rate or carcass weight between lambs fed different diets. Similarly, no effect of adding protected CLA on the weight, depth and width of LD was observed. Also Gillis *et al*. [2004] feeding beef cattle with rumen-protected CLA reported no significant effect on carcass parameters. Gassman [2000], in turn, did not note any influence of Ca CLA salts on LD area in crossbred finishing steers. Cook *et al*. [1998], Thiel-Cooper *et al*. [2001] and Wiegand *et al*. [2001] reported no effect of dietary CLA on the loin muscle area in pigs.

An addition of IGO reduced, however, lambs adiposity (backfat thickness, subcutaneous and intermuscular fat in leg) and the decreased fat content in the muscular tissue (intramuscular fat in LD and MS) – Tabela 3.

The enrichment of the lamb diet with IGO reduced the thickness of subcutaneous fat over loin eye by 24.5% ($P \leq 0.01$) and the amount of subcutaneous and intermuscular fat in leg by 19.1% ($P < 0.05$) and 23.5% ($P < 0.01$), respectively. Moreover, the IGO supplement lowered the fat content of meat. The content of intermuscular fat fell by 22.2 percentage points (pp) – ($P \leq 0.01$) – for *M. longissimus dorsi* and by 17.1 pp for *M. semitendinosus* ($P \leq 0.05$).

The fact that dietary CLA has a beneficial effect on body fat reduction is also in accordance with studies on the other animals [Azain *et al*. 2000, Park *et al*. 1999, Sisk *et al*. 2001]. For example, rodents fed 1-1.5% CLA as a crude mixture of *c*9*t*11 and *t*10*c*12 showed less body fat and greater lean body mass than control animals [DeLany *et al*., 1999, Park *et al*. 1999, Tsuboyama-Kasaoka *et al*. 2000, West *et al*. 1998]. The addition of CLA to mouse and rat diets reduced their adipose tissue by 55% and 23%, respectively [Pariza *et al*. 1997]. Meanwhile, Park *et al*. [1997] reported a reduction in adipose tissue by as much as 60% after 4 weeks of supplementing mice diet with CLA. Feeding Sprague-Dawley rats with 0.25-0.5% of a crude mixture of CLA isomers for 5 weeks reduced retroperitoneal and parametrial fat [Azain *et al*. 2000]. Feeding 0.05-1.0% of mixed CLA isomers to pigs reduced backfat thickness without affecting total body weight [Cook *et al*. 1999]. Similarly, feeding 0.07-0.5% of mixed CLA isomers to growing pigs for 8 weeks increased feed efficiency and lean body weight while reducing fat deposition as compared to controls [Ostrowska *et al*. 1999, 2003]. Similarly, in barrows fed 0,12 to 1,0% CLA intramuscular lipids were found significantly reduced [Thiel-Cooper *et. al*. 2001]. Wiegand *et al*. [2001] observed decreased backfat thickness in barrows fed 0.75% dietary CLA.

Limited information is available upon the effect of CLA on body fat in ruminants. Gassmann *et al*. [2000] reported a numeric decrease in *subcutaneous* fat thickness

measurements in steers fed with CLA. Sinclair *et al*. [2010] using CLA addition in lactating ewes noted reduced backfat thickness between the 10th and 11th thoracic vertebra. Conjugated linoleic acid *trans*-10,*cis*-12 has also been related to a reduction in milk fat content of dairy cows [Baumgard *et al*. 2001] and lactating ewes [Lock *et al*. 2006].

Not all studies, however, confirm such CLA activity. Wynn *et al*. [2006] adding CLA to sheep diet in a form of calcium salts, did not confirm their adiposity decrease. Similarly, in beef cattle fed rumen-protected CLA it did not alter carcass or kidney and pelvic fat content on carcass [Gills *et al*. 2004]. Gassman [2000] did not observe any influence of Ca salts of CLA fed to crossbred finishing steers on the thickness of backfat. Neither was any CLA influence observed on body fat content in the studies by Eggert *et al*. [2001] and Demaree *et al*. [2002].

The mechanism by which CLA reduces adipose tissue is not fully understood. Numerous studies have suggested that CLA increases energy expenditure as shown by increasing oxygen consumption [Choi *et al*. 2004] or by increased expression of uncoupling proteins [Ealey *et al*. 2002]. This mechanism may also rely on the reduction of adipose cell mass and / or cell numbers by inhibiting lipoprotein lipase at adipose cells [Lin *et. al*. 2001], by inhibiting stearoyl-CoA desaturase activities [Ntambi *et al*. 1999], by enhancing apoptosis of preadipocytes and adipocytes [Tsuboyama-Kasaoka *et al*. 2000] or by modulating adipokines and cytokines [Akahoshi *et al*. 2002]. This phenomenon may also be explained by increased fatty acid β-oxidation in skeletal muscle.

It is therefore clear that no conclusive explanation exists why CLA or its metabolites reduce the fat content of body and the findings of various authors only suggest the possible course of these processes and are not always confirmed by other studies [Simon *et al*. 2005].

It is now known that the effect of CLA on adipogenesis and fat metabolism in animals depend on age, ration, type of isomer, duration of treatment, and animal species [Evans *et al*. 2002]. Reductions in fat content of mice are generally greater then those found in other species [Azain *et al*. 2000]. Of all the conjugated dienes of linoleic acid identified, the isomer of *trans*-10,*cis*-12 configuration is most responsible for reducing fatness [Brown *et al*. 2001, DeLany *et al*. 1999, Park *et al*. 1997]. Rodriguez *et al*. [2002] demonstrated that while *trans*-10,*cis*-12 CLA treatment at doses to those found in serum from rodents reduced adipogenesis and lipid droplet accumulation, *cis*-9,*trans*-11 isomer had opposite effects in primary cultures of brown adipocytes. They also reported that effects of *trans*-10, *cis*-12 CLA were predominant over the effects of *cis*-9,*trans*-11. A large number of studies in animal models have also been performed using CLA mixtures with different isomer ratios, others using isolated isomers have provided a great deal of evidence to suggest that the biologically active isomer with anti-obesity effects is *trans*-10,*cis*-12 [Park *et al*. 1999, Sinclair *et al*. 2010, Wang and Jones 2004].

In the present study, fattening lambs were supplemented with isomerised grapeseed oil that was a mixture of CLA isomers of *cis*-9,*trans*-11 (~49%), *trans*-10,*cis*-12 (~46%) and *cis*-11,*trans*-13 (~5%) configuration. Probably the high proportion of *trans*-10, *cis*-12 isomer in the supplement used (its daily consumption by lambs from the experimental group was about 6,5 g/animal) is responsible for a significant decrease in lamb fatness.

The results of this study demonstrate that isomerised grapeeseed oil enriched with CLA added to feedlot lamb diets did not alter animal growth and carcass weight.

The administration of this supplement to fattening lambs reduced their fatness (subcutaneous and intermuscular fat) from 19 to 24% and fat content of their meat from 17 to 22 pp. The reduction in intramuscular fat content is significant in so far as this fat cannot be removed during culinary treatment of meat.

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