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EFFECT OF SUNFLOWER OIL OR CLA ADDITION ON α-TOCOPHEROL STATUS AND LIPID OXIDATION IN PORK*

Marek Pieszka[1], Katarzyna Połtowicz[2], Tadeusz Barowicz[1], Mariusz Pietras[1]

[1]Department of Animal Nutrition and Feed Science, [2]Poultry Breeding Department; National Research Institute of Animal Production, Kraków

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The substitution of 2% sunflower oil by the same quantity of CLA preparation (EDENOR UKD 6010, Henkel, Germany) in fattener diets caused a change in fatty acid composition of the *longissimus* muscle, mainly in the palmitic and stearic acids from 28.7 to 29.25% and from 10.4 to 11.5%, respectively, of the total fatty acids. Lipid saturation was accompanied by a significant decrease in PUFA and the narrowing of *n-6*/*n-3* acid rates ($p<0.05$). The decrease in PUFA level concerned mainly linolic, linolenic and arachidonic acid: from 15.9 to 12.1%; from 1.12 to 0.99% and from 1.14 to 0.87 % respectively. In the lipids of the *longissimus* muscle, a significant increase was noted in CLA: from 0.21 to 1.11% of total fatty acids ($p<0.01$). A significant decrease in α-tocopherol from 3.64 to 1.54 μg/g was stated for pigs obtaining CLA in the diet ($p<0.01$).

The CLA administered to the diet was found to affect the process of fat oxidation (TBARS value) in the *longissimus* muscle samples stored for 60 days at -19°C. Experimental factors used in this study did not deteriorate the physicochemical traits, however, an increase in crude fat content of the *longissimus* muscle by 1.13% was observed. The tendencies for lower values of L*a*b* in the *longissimus* muscle of fatteners receiving the CLA-supplemented the diet was observed.

The change in fatty acid profile of the *longissimus* muscle and the increase in saturated fatty acid levels were noted as a result of feeding the CLA addition to fatteners. Moreover, the meat colour during storage at low temperatures was more stable.

INTRODUCTION

The feeding of fatteners in the last weeks of life has a significant influence on the carcass intramuscular fat value and technological usability of meat. Feed mixtures for pigs, mainly for fatteners, are prepared in the fattening process for an increase in metabolizable energy from 11.5 to 13.5 MJ. A higher energy supply in the form of fatty acids, especially their unsaturated forms existing mainly in vegetable oils, could cause an increase in meat lipid tractability for cell oxidation processes [Joo *et al.*, 2002]. The changes caused by fat oxidation in the muscular tissue are the main reasons for undesirable chemical and sensory changes in both the unprocessed meat and prepared products [Buckley *et al.*, 1995]. Moreover, the introduction of vegetable oils into the feed mixtures for pigs causes an increase in antioxidant vitamin demand especially vitamin E [Monahan *et al.*, 1993]. Conjugated linoleic acid (CLA), with its especially valuable traits, shows a wide spectrum of activity in the body [Pariza *et al.*, 1996]. CLA denotes the mixture of positional and geometrical isomers. The most active forms are: *9 cis*, *11 trans* and *10trans*, *11 cis* of linoleic acid – C 18:2. When administered to diets for fatteners, CLA influences fatty acid metabolism changes and their profile, however, it can have a disadvantageous effect on the physicochemical traits of meat [Ostrowska *et al.*, 1999; Joo *et al.*, 2002]. Moreover, Du *et al.* [2000] suggested that CLA indirectly increases the oxidation stability of meat by increasing saturated fatty acid levels while simultaneously decreasing unsaturated fatty acids levels and also inhibits the production of free radicals. The CLA does not demonstrate any antioxidative traits [Livisay *et al.*, 2000].

The aim of this study was to evaluate a degree of lipid oxidation, vitamin E status and selected physicochemical traits of meat of pigs fed in the final period of fattening with a mixture in which sunflower oil was replaced by the equivalent quantity of CLA preparation.

MATERIAL AND METHODS

The study was carried out on 24 cross fatteners [♀(♀ Large Polish White x ♂ Polish Landrace) x ♂ Pietrain] of an average body weight of 70 kg, divided into 2 groups, 12 animals each. Fatteners were kept in individual pens and fed according to Polish Nutrient Requirement [1993], with pelleted feeds containing among others: 14.2% of total protein, 6.5% of raw fat, 12.68 MJ of metabolic energy and 7.8 g of lysine. Fatteners of the experimental group received an addition of CLA (EDENOR UKD 6010, Henkel, Germany) in the amount of a 2% of a daily ration. The diet of the second group was supplemented with 2% sunflower oil to maintain the same energy value of both diets. The fatty acid content of the feed mixture and supplements used in this study are shown in Table 1. The daily feed doses were

Author's address for correspondence: Marek Pieszka, Department of Animal Nutrition, National Research Institute of Animal Production, ul. Krakowska 1, 32-083 Balice, Poland; tel.: (48 12) 255 81 84; fax: (48 12) 258 81 14; e-mail: pieszkam@wp.pl

given in two rations and animals had a free access to water. The study was concluded with slaughtering after the fatteners achieved 100 kg of body weight. Immediately following dissection, samples of the *longissimus* muscle from the last pectoral vertebra and the first lumbar vertebra area were taken. Meat samples without coat and fascias were mixed in Moulinette (Spain) and then samples were placed into PCV boxes and stored at -19°C for later analyses. The chemical composition of feed mixtures was evaluated using AOAC standard methods [1995]. Meat samples were determined for the chemical composition [Budsławski & Drabent, 1972] and pH - measured in water homogenates according to Polish Norm PN-77/A-82058 [1977]. Meat colour was evaluated on a CIE scale after 60 days of storage: L*a*b* were measured using a colorimeter (Minolta CR-310, Tokyo, Japan) according to standard methods [CIE, 1976]. Water--holding capacity was evaluated according to the method of Grau and Hamm [1953]. Chromatographic analysis of vitamin E in the *longissimus* muscle was carried out using the HPLC method, after saponification and extraction with ethyl acetate and a hexane mixture [Ueda & Igarashi, 1987]. Fatty acid composition (including CLA) was determined using gas chromatography. Lipids were extracted from feed and meat using the method of Folch *et al.* [1957]. In the samples of the *longissimus* muscle, the TBARS value was evaluated according to the method described by Pikul [1993] after 60 days of storage at a temperature of -19°C.

All results were statistically verified using a single-factor variation analysis.

TABLE 1. Nutritive value and fatty acid composition (the % total fatty acids) of complete feed mixture and fatty additions used in this study.

Components	Complete feed mixture and fatty additions		
Total protein (%)	14.2		
EM (MJ)	12.68		
Crude fibre (%)	6.50		
Lysine (%)	0.78		
Methionine (%)	0.25		
Ca (%)	0.55		
P (%)	0.48		
Fatty acid composition (%)	Mixture	Sunflower oil	CLA*
UFA	82.41	88.80	93.10
MUFA	26.15	24.70	29.80
PUFA	56.26	64.10	63.30
PUFA *n-6*	48.52	64.00	63.30
PUFA *n-3*	7.74	0.10	-
PUFA *n-6*/*n-3*	6.27	-	-
CLA**	-	0.10	61.30

*ENDENOR UKD (Henkel Oleochemicals, Germany); ** CLA contained the following isomers: C 18:2 *tt* – 0.8%; C 18:2 *c9t11* – 9.1%; C 18:2 *t8c10* – 9.5%; C 18:2 *c11t13* – 10.5%; C 18:2 *t10 c12* – 10.2%; C 18:2 *cc* – 21.2%.

RESULTS AND DISCUSSION

The introduction of CLA to the diet caused a change in the fatty acid profile of *longissimus* muscle lipids. The meat of fatteners receiving CLA in their diet was characterised by a 5-fold increase in CLA content ($p<0.01$) compared with the group fed with a sunflower oil addition (Table 2). The results obtained are consistent with those reported by other authors. Tischendorf *et al.* [1999] described the conversion of CLA from a mixture to fat, meat and liver lipids, while showing that there is a linear dependence between particular CLA isomer concentrations in mixture and tissues. In addition, Thiel-Cooper *et al.* [2001] observed the linear dependence between CLA in a mixture and its level in meat lipids and in back fat. In this study, the lipids of the *longissimus* muscle of pigs fed a CLA-containing mixture were more saturated compared to pigs of a group fed with the addition of sunflower oil. The saturation differences concerned mainly the palmitic and stearic acids.

TABLE 2. Fatty acid composition (the % of total fatty acids) of the *longissimus* muscle (n=12).

Fatty acids	Groups		SEM
	Sunflower oil	CLA	
C 12:0	0.12	0.13	0.01
C 14:0	1.92	1.95	0.10
C 16:0	28.75	29.20	0.55
C 16:1	2.34	2.28	0.14
C 18:0	10.37	11.53	0.41
C 18:1	37.70	39.45	0.65
C 18:2	15.96	12.09	0.85
CLA	0.21^{A}	1.11^{B}	0.18
C 18:3	1.12	0.99	0.05
C 20:0	0.0	0.05	0.02
C 20:4	1.14	0.87	0.07
EPA	0.08	0.09	0.003
DHA	0.03	0.03	0.01
Others	0.26	0.23	0.05
SFA	41.33	43.02	0.63
UFA	58.67	56.98	0.62
MUFA	40.04	41.73	0.68
PUFA	18.63^{a}	15.25^{b}	0.81
n-3 PUFA	1.23	1.10	0.06
n-6 PUFA	17.1^{A}	13.03^{B}	0.66
n-6/*n-3* PUFA	13.90^{a}	11.83^{b}	0.59

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 ($p<0.01$).

Significant differences in the unsaturated fatty acids level of fat tissue between experimental and control animals were not observed. However, pigs fed with a CLA addition had less C16:1, C18:2, C18:3 and C20:4 acids compared to pigs fed with a sunflower oil addition. Similar results were achieved in studies using mice as experimental animals [Belury & Kempa-Steczko, 1997], where a decrease in C18:1 and C20:4 acids in the neutral lipids of the liver was observed upon 1.5% CLA addition to the diet. Banni *et al.* [1999] also observed that CLA addition to a rat diet decreased the level of C18:3, C20:3 and C20:4 acids in mammary gland lipids.

In this study, a significant decrease in PUFA content was observed ($p<0.05$). The proportion of *n-6*/*n-3* PUFA acids was narrowed in a positive direction from the point of view of modern human dietetics ($p<0.05$), mainly because of the significant decrease in *n-6* PUFA share in total fatty acids.

PUFA *n-3* contents were slightly lowered. Hayek *et al.* [1999] stated that a 1% CLA addition to a rat diet decreased the *n-3* acid levels in neutral lipids.

The 2% addition of CLA to the fattener diet caused a decrease in α-tocopherol content ($p<0.01$) in the *longissimus* muscle. This could suggest that an increase in vitamin E with a CLA addition is a result of α-tocopherol share in the stabilization of the double binding of CLA, inbuilt in the phospholipid complex of the cell membrane. A similar conclusion was obtained in a study on rats where the degree of lipid oxidation and α-tocopherol status were evaluated in liver microsomes and muscle homogenates [Livisay *et al.*, 2000]. For this reason, an increase in the vitamin E dose in the fattener's mixture containing CLA is recommended [Waylan *et al.*, 2002].

Lipid oxidation degree was evaluated by marking the TBARS number of meat substances reacting with TBA. The effect of CLA on the malonaldialdehyde (MDA) level was not stated (Table 3). MDA is mainly formed during the oxidation of fatty acids containing three or more double bindings, where MDA content increases together with an increase in the number of double bindings [Janero, 1990]. In this experiment, it was established that CLA given in the diet decreased the levels of some polyenic acids – C18:3, C20:4. The higher value of TBARS numbers in the experimental group was likely to result from the higher (by nearly 1.13%) total raw fat content in the *longissimus* muscle. In other studies, the stabilizing influence of CLA on chicken and pigs meat lipid oxidation was found by Du *et al.* [2000] and Joo *et al.* [2002], respectively.

TABLE 3. The effect of CLA addition on physicochemical traits, α-tocopherol and TBARS content of the *longissimus* muscle samples after storage at –19°C for 60 days (n=12).

Fatty acids	Groups		SEM
	Sunflower oil	CLA	
Dry matter (%)	27.10	27.58	0.26
Crude fat (%)	3.20	4.33	0.34
Crude protein (%)	22.61	22.03	0.21
pH_{24}	5.54	5.48	0.04
Water-holding capacity (%)	24.69	24.88	1.11
Colour of meat:			
L*	53.47	51.68	0.93
a*	8.44	6.66	0.62
b*	17.25	16.90	0.23
α-tocopherol (g/g)	3.64^{B}	1.54^{A}	0.13
TBARS (mg/kg)	0.196	0.264	0.035

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 ($p<0.01$)

No significant differences in the physicochemical traits of meat: pH, colour, water-holding capacity and chemical composition, were found in this study. However, the tendency towards a higher ether extract content of the *longissimus* muscle was observed for animals obtaining the CLA addition. In other studies, higher than 1% doses of CLA caused an increase in the intramuscular fat content of meat [Dugan *et al.*, 1999]. CLA addition to the diet did not cause any significant changes in the colour of meat stored at low temperatures for 60 days (Table 3). The tendency for lower L*a*b* value of *longissimus* muscle for fatteners obtaining conjugated linoleic acid was observed, which supports its influence on the oxidative stabilization of intramuscular fat. Similar results were obtained by O'Quinn *et al.* [2000] who added 0.5% CLA to diets for fatteners. Colour stability seems to be linked to lipid oxidation by changing the fatty acid composition, inhibition of pigment oxidation and the opportunity of its longer storage.

CONCLUSIONS

1. The introduction of 2% CLA to fattener diets caused a 5-fold increase in this acid in meat ($p<0.01$), an increase in the degree of fatty acid saturation, and a decrease in PUFA content, especially from the *n-6* family ($p<0.01$). As a consequence, the proportion of PUFA from *n-6*/*n-3* families was narrowed.

2. CLA introduced to fattener diets decreased the α-tocopherol content ($p<0.01$) of the *longissimus* muscle.

3. CLA added in the nourishment dose did not affect the physicochemical traits of the *longissimus* muscle nor the fat oxidation processes (TBARS value).

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WPŁYW PODAWANIA OLEJU SŁONECZNIKOWEGO LUB SPRZĘŻONEGO KWASU LINOLOWEGO (CLA) NA STATUS α-TOKOFEROLU I OKSYDACJĘ LIPIDÓW W MIĘSIE WIEPRZOWYM

Marek Pieszka[1], Katarzyna Połtowicz[2], Tadeusz Barowicz[1], Mariusz Pietras[1]

[1]Zakład Paszoznawstwa; [2]Katedra Hodowli Drobiu, Instytut Zootechniki w Krakowie

Zastąpienie (2%) oleju słonecznikowego równoważną ilością preparatu CLA (EDENOR UKD 6010, Henkel, Niemcy) w diecie tuczników spowodowało zmianę składu kwasów tłuszczowych w mięśniu najdłuższym, głównie kwasu palmitynowego i stearynowego z wartości odpowiednio: 28,7 do 29,2% oraz 10,4 do 11,5% sumy kwasów (tab. 2). Nasyceniu lipidów mięśnia najdłuższego towarzyszyło istotne obniżenie PUFA i zawężenie proporcji kwasów PUFA z rodziny *n-6*/*n-3* ($p<0,05$). Zmniejszenie zawartości PUFA dotyczyło głównie kwasu linolowego, linolenowego i arachidonowego z wartości odpowiednio: 15,9 do 12,1%; 1,12 do 0,99% i 1,14 do 0,87%. W lipidach mięśnia najdłuższego nastąpił istotny wzrost poziomu CLA, z wartości 0,21 do 1,11% sumy kwasów ($p<0,01$). Stwierdzono istotne obniżenie poziomu α-tokoferolu z 3,64 do 1,54 μg/g w mięsie u świń otrzymujących CLA ($p<0,01$). Nie obserwowano wpływu podawanego CLA na wartość TBARS w próbkach mięśnia najdłuższego przechowywanego przez 60 dni w temperaturze -19°C (tab. 3) Zastosowany czynnik doświadczalny nie pogarszał istotnie cech fizykochemicznych, chociaż w mięśniu najdłuższym w grupie doświadczalnej obserwowano 1,13% wzrost zawartości tłuszczu surowego. Stwierdzono tendencje do niższych wartości L*a*b* mięśnia najdłuższego u tuczników otrzymujących CLA.

W wyniku podawania paszy z zawartością CLA nastąpiła zmiana profilu kwasów tłuszczowych lipidów w mięśniu najdłuższym oraz wzrost poziomu kwasów nasyconych. Ponadto podczas przechowywania mięsa w niskiej temperaturze jego barwa była stabilniejsza.