Journal of Animal and Feed Sciences, 15, 2006, 37–45

The effect of sex and dietary antioxidants β-carotene, vitamins C and E in a CLA-enriched diet on the lipid profile and oxidative stability of pork meat*

M. Pieszka[1,6], P. Paściak[2], A. Janik[1], T. Barowicz[1], D. Wojtysiak[4] and W. Migdał[5]

[1]National Research Institute of Animal Production, Department of Nutrition and Feed Science
Krakowska 1, 32-083 Balice, Poland
[2]Ecopig, 42-510 Wojkowice Kościelne 28, Poland
[4]Agricultural University of Cracow, Department of Anatomy
Al. Mickiewicza 24/28, 30-059 Kraków, Poland
[5]Agricultural University of Cracow, Department of Animal Products Technology
Balicka 122, 31-149 Kraków, Poland

(Received 9 March 2005; revised version 24 October 2005; accepted 6 January 2006)

ABSTRACT

Fifty Polish Large White growing pigs were randomly divided into 5 groups (5 gilts and 5 barrows in each group) and fattened from 50 to 105 kg body weight. The experimental factors were gender and addition of vitamins C, E and β-carotene to the diets. All diets were supplemented with 0.5% CLA. Polyunsaturated fatty acids (PUFA) were observed to decrease in pigs receiving combined vitamins C, E and β-carotene compared with pigs receiving a single supplement of β-carotene ($P<0.05$). In gilts the level of saturated fatty acids (SFA) was significantly lower and the content of unsaturated fatty acids (UFA) and PUFA was significantly higher than in barrows ($P<0.01$). A high significant difference ($P<0.01$) was also found between gilts and barrows in the PUFA/SFA ratio. The CLA concentration was significantly higher in gilts than in barrows ($P<0.05$). Highly significant sex-dependent differences were found in the content of crude fat, which was significantly lower ($P<0.01$) in gilts than in barrows. The pH of meat 24 h *post-mortem* was 5.44 in gilts and 5.55 in barrows ($P<0.01$). Yellowness (b*) was found to decrease in all experimental groups compared with the control group ($P<0.01$). There was a tendency towards a lower concentration of tiobarbituric acid reactive substances (TBARS) after 3-month storage of frozen meat in the group receiving supplemental vitamin E and combined vitamins C, E and β-carotene compared with the group receiving β-carotene alone ($P<0.05$). The vitamin supplements caused significant changes in the vitamin E content of meat

* Supported by the Ministry of Scientific Research and Information Technology, Grant No. P06Z 052 24
[6] Corresponding author: e-mail: mpieszka@izoo.krakow.pl

(P<0.01): the highest concentration (3.06 μg/g) was found in fatteners supplemented with combined vitamins E, C and β-carotene. The vitamin supplements, including β-carotene, exerted no influence on the vitamin A concentration in meat. No significant interaction between gender and the studied supplements was found.

KEY WORDS: β-carotene, vitamin C, vitamin E, CLA, pigs, meat

INTRODUCTION

Conjugated linoleic acid (CLA) is a term that refers to a mixture of positional isomers of octadecadienoic acid with conjugated double bonds. CLA increases the metabolic rate and promotes muscle growth while reducing body fat (Müller et al., 2000). Dietary CLA given to pigs at a rate of 0.5-2% has the greatest effect on changing the metabolism of fatty acids by limiting carcass fatness, but its excessive concentration in the tissues may adversely affect the physicochemical properties of meat (Joo et al., 2002; Ostrowska et al., 2003). These unfavourable processes can be counteracted by antioxidants, preferably natural ones, the most important of which are α-tocopherol, ascorbic acid and β-carotene, which may prevent and counteract the effects of lipid peroxidation in meat (Monahan et al., 1993). Vitamin E is the first line of defense against peroxidation of PUFA in the cellular lipid phase (Ohshima et al., 1993).Vitamin C is an essential component bound to the aqueous phase, protecting cell structures against malfunction *via* free radical scavenging (Banhegyi et al., 1997). In that way vitamin C counteracts lipid peroxidation and may decrease the synthesis of cytotoxic products, especially TBARS-thiobarbituric acid reactive substances (Sawosz et al., 2005). Moreover, vitamin C takes part in reconversion of vitamin E, allowing more effective use of the tocopherol taken up from the diet (Packer, 1991). Carotenoids are very widespread pigments in nature, found especially in various fruits and other plant parts. Their structure consists of conjugated doubled bonds that are related to active oxidative radicals. β-caroten is considered to have one of the highest activities. Some studies show that interactions could exists between carotenoids and tocopherols, carotenoids and ascorbic acid (Hof et al., 1999). It was found that tocopherols could protect β-caroten during induced lipid peroxidation with free radicals. Such caroten-tocopherol interactions were shown in a membrane model in which combinations of α-tocopherol with β-caroten significantly inhibited peroxidation of lipids. Their joint influence was synergic (Palozza and Krinsky, 1992). The above vitamins, through their effect on different cell structures, complement each other in their oxidative function.

Sex differences have also been examined by several authors in respect to pork fatty acid composition (Hartmann et al., 1997; Hanczakowska, 2004). The fatty

acid profile of gilts in comparison with barrows was very often characterized by a higher concentration of PUFA.

The aim of this study was to evaluate the effect of gender and dietary antioxidants, i.e. β-carotene, vitamins C and E, in a CLA-enriched diet on the lipid profile and oxidative stability of pork meat.

MATERIAL AND METHODS

Animals, treatments and experimental procedure

Fifty Polish Large White fatteners were randomly divided into 5 groups (5 gilts and 5 barrows in each group). They were fattened from 50 to 105 kg body weight using diets with different antioxidants: β-carotene, vitamins C and E (Table 1). Due to instability of ascorbic acid and α-tocopherol, both vitamins

Table 1. Content of vitamins E[1] and C[2] and β-carotene in the premixes made using Lutamix complete, BASF (Kutno, Poland) as the base

Dietary treatments
Group I – basal diet + 0,5% CLA + standard premix (30 mg vitamin E/kg feed)
Group II – basal diet + 0,5% CLA + experimental premix (200 mg β-carotene /kg feed)
Group III – basal diet + 0,5% CLA + experimental premix (200 mg vitamin C/kg feed)
Group IV – basal diet + 0,5% CLA + experimental premix (300 mg vitamin E/kg feed)
Group V – basal diet + 0,5% CLA + experimental premix (200 mg β-carotene, 200 mg vitamin C, 300 mg vitamin E/kg feed)

[1] vitamin E was provided as α-tocopherol acetate

[2] vitamin C was provided as sodium ascorbate

were provided in the form of sodium ascorbate and α-tocopherol acetate, respectively, in the premixes. All diets contained a 0.5% CLA supplement (Edenor UKD 6010, Henkel). CLA consisted of the following isomers: C 18:2 *c9t11*-20.9%, C 18:2 *t10c12*-22.3%, C 18:2 *c9c11*-18.5%, C 18:2 *t9t11*-6.7%. The metabolizable energy content was 13.0 MJ/kg, and its level was calculated from the composition of the diet, assuming tabular values for individual components given in the Nutrient Requirements of Pigs (1993). The composition, nutritive value and fatty acid content in the complete mixture are given in Table 2. All animals were kept in individual pens with free access to water. The mixture was individually administered *ad libitum* into an automatic feeder. After reaching 105 kg of body weight all fatteners were slaughtered. Dissection was carried out 24 h after slaughter after cooling the carcasses at 4°C. Samples of *longissimus dorsi* muscle were taken from the area of the last thoracic vertebra and first lumbar vertebra. The samples were then frozen at -19°C.

Table 2. The composition, nutritive value and fatty acid composition (% of total fatty acids) of the complete mixture

Item	Composition, %
Ingredient, %	
ground wheat	44.5
ground triticale	20
ground maize	10
soyabean meal	21
limestone	1.5
dicalcium phosphate	0.5
CLA	0.5
vitamin/mineral premix (Lutamix complete, BASF) [1]	2
Nutritive value per kg as fed, %	
metabolizable energy , MJ	13.0
crude protein	17.0
crude fibre	3.64
crude fat	2.09
crude ash	2.58
Ca	0.85
total P	0.52
Na	0.13
Lys	0.93
Met and Cys	0.59
Thr	0.61
Try	0.19
Fatty acid composition, %	
UFA	82.41
MUFA	26.15
PUFA	56.26
PUFA *n-6*	48.52
PUFA *n-3*	7.74
PUFA *n-6/n-3*	6.27

[1] see Table 1

Analytical methods

The basic and amino acid compositions of the diets were determined using standard methods (AOAC, 1995). In meat samples, the chemical composition was evaluated according to Budsławski and Drabent (1972). Meat samples were ground in a kitchen mixer (Moulinette, Spain), transferred into polyvinyl chloride boxes and placed in cold storage at -19°C for further analyses. Meat colour in the L*a*b* system (CIE, 1976) was evaluated using a Minolta Chromameter CR-310 (Japan), 24 h *post-mortem* on a 2.5 cm thick slice of LD after one h of exposure to light. The pH was measured using a pH meter (Matthäus, Germany) with a glass

electrode standardized for pH 4.01 and 7.0 according to Polish Standard PN-77/a-82058. Water holding capacity was evaluated according to the Grau and Hamm method (1953). The composition of essential fatty acids in meat was analysed by gas chromatography (Varian, USA) after previous extraction of lipids according to the method of Folch et al. (1957). α-tocopherol and retinol were analysed by HPLC (Merck-Hitachi, Germany) according to a modified method of Ueda and Igarashi (1987). In samples of *longissimus dorsi* muscle, the TBARS value was evaluated according to the method described by Salih et al. (1987) after 90-day storage at -19˚C.

The results obtained were analysed statistically with two-way analysis of variance ANOVA and Tukey's test using the computer program, Statgraphics Plus 4.0.

RESULTS AND DISCUSSION

The results of the experiment are shown in Tables 3 and 4. Analysis of the composition of fatty acids in the *longissimus dorsi* muscle showed a

Table 3. Fatty acid composition of *M. longissimus dorsi* in pigs receiving different supplements of vitamins

Fatty acids	Diets					Sex		SEM	Interaction diet × sex[1]
	I basal	II β-car.	III vit. C	IV vit. E	V β-car, C,E	gilts	barrows		
SFA	41.60	41.36	41.93	41.64	42.36	40.48^{A}	43.07^{B}	0.27	NS
UFA	58.63	58.39	58.06	58.35	57.63	59.51^{B}	56.92^{A}	0.27	NS
MUFA	41.24	39.95	41.89	40.92	42.49	40.49	41.90	0.35	NS
PUFA	17.38^{ab}	18.43^{b}	16.17^{ab}	17.42^{ab}	15.14^{a}	18.81^{B}	15.01^{A}	0.45	NS
PUFA/SFA	0.42	0.44	0.39	0.42	0.36	0.46^{B}	0.35^{A}	0.01	NS
PUFA*n-6*/*n-3*	45.5	46.3	40.9	44.8	40.4	44.4	42.8	0.83	NS
Sum of CLA	1.33	1.43	1.34	1.28	1.31	1.38^{b}	1.29^{a}	0.2	NS

$^{a, b}$ - $P<0.05$; $^{A, B}$ - $P<0.01$; [1]NS - $P>0.05$

tendency towards a lower concentration of polyunsaturated fatty acids (PUFA) in the group of pigs receiving supplemental vitamins C, E and β-carotene (group V) in comparison with group II ($P<0.05$) of fatteners receiving only β-carotene. These results could be explained by the co-operative interaction among these compounds, especially vitamins C and E. Several studies demonstrate that interactions can occur between carotenoids and tocopherols, carotenoids and ascorbic acid (Handelaman et al., 1991; Hof et al., 1999). Significant differences were found in the composition of fatty acids depending on sex. In gilts, the concentration of saturated fatty acids (SFA) was significantly lower and the concentration of unsaturated fatty acids (UFA), especially PUFA, was significantly higher ($P<0.01$) than in barrows.

Table 4. The physicochemical and sensory traits of fatteners receiving CLA and vitamins

Item	Diets					Sex		SEM	Inter-action diet x sex
	I basal	II β-car.	III vit. C	IV vit. E	V β-car. C, E	gilts	barrows		
Dry matter,%	26.2	26.1	26.6	26.1	27.0	26.1^{a}	26.7^{b}	0.14	NS
Crude protein, %	23.6	23.2	23.7	23.4	23.8	23.6	23.4	0.09	NS
Crude fat, %	2.07	2.39	2.35	2.02	2.73	1.96^{A}	2.66^{B}	0.10	NS
pH_{45min}	6.70	6.91	6.86	7.00	6.91	6.91	6.86	0.03	NS
pH_{24h}	5.45	5.43	5.49	5.57	5.55	5.44^{A}	5.55^{B}	0.02	NS
Water holding capacity, %	32.4	31.8	28.6	29.1	31.9	29.8	31.7	0.56	NS
Thermal losses, %	32.2	33.4	32.0	32.0	32.9	32.8	32.2	0.42	NS
Colour of meat:									
[1]L*	54.2	53.9	54.8	54.5	54.5	54.3	54.5	0.24	NS
a*	15.3	15.2	14.8	14.6	15.2	14.9	15.2	0.13	NS
b*	6.8^{B}	5.0^{A}	5.2^{A}	5.2^{A}	5.3^{A}	5.4	5.5	0.12	NS
TBARS, mg/kg^{-1}	0.449^{ab}	0.507^{b}	0.471^{ab}	0.411^{a}	0.424^{a}	0.428	0.476	0.01	NS
α-tocopherol, μg/g	1.23^{A}	1.13^{A}	1.76^{B}	2.88^{C}	3.06^{C}	2.03	1.99	0.11	NS

[1] L* - lightness (higher values indicate a lighter colour); a*- redness (higher values indicate a redder colour); b*- yellowness (higher values indicate a more yellow colour)

Differences in the composition of fatty acids between gilts and barrows were reported by Hanczakowska (2004) for fatteners receiving higher supplements of vitamins E, C and β-carotene, which may be evidence that the metabolism of SFA and PUFA differs and depends on the animals' sex. It is assumed that this difference is connected to different activity of those enzymes in animal tissues that are responsible for the synthesis of particular classes of fatty acids. One of them is acetyl-CoA-carboxylase (CBX), which catalyses the first step in the fatty acid biosynthetic process. This enzyme has a key role in the regulation of fatty acid biosynthesis in animal tissues and is generally considered to be a rate-limiting enzyme of lipogenesis in animals (Numa et al., 1970), especially pigs (Mersmann et al., 1973).

The dietary supplement of β-carotene and vitamins C and E had no significant effect on the contents of dry matter, crude protein and fat or on the value of pH_{45min}, pH_{24h}, water holding capacity, thermal losses and colour of meat (Table 4). Meat colour measured 24 h *post-mortem* in the L*a*b* system indicates only that the high supplement of β-carotene and vitamins C and E reduced yellowness (b*) ($P<0.01$) in groups II, III, IV and V in comparison with group I. In the studies of Monahan et al. (1993) and Pieszka et al. (2004), the dietary supplement of vitamin E improved meat colour and stability. This effect is attributed to the inhibiting influence of vitamin E on the oxidation of myoglobin, which makes meat red in contradistinction to its oxidized form, metmyoglobin, which makes

meat brown. Other authors (Zanardi et al., 1999) observed no effects of vitamin E supplementation on the colour of fresh pork.

The TBARS content in the meat of pigs supplemented with vitamin E (group IV-0.411 mg TBARS/kg^{-1}) and β-carotene combined with vitamins E and C (group V-0.424 mg TBARS/kg^{-1}) was significantly lower than TBARS in group II (0.507 mg TBARS/kg^{-1}), which received only supplemental β-carotene.

The inhibitory effect of vitamins E, C and β-carotene on lipid oxidation in pigs was shown, among others, by Hanczakowska (2004). In this trial, a higher concentration of TBARS was found in the meat of fatteners receiving β-carotene (group II) compared with the meat of pigs receiving vitamin E (group IV), and β-carotene combined with vitamins C and E (group V), which may attest to the weaker antioxidative activity of β-carotene in comparison with vitamins E and C. This is attributed to the fact that this activity is correlated to the vitamin E (α-tocopherol) content of meat. According to this, the highest concentration of vitamin E in meat was found in groups IV (2.88 μg/g) and V (3.06 μg/g) and the lowest in group II (1.13 μg/g). Also vitamin C supplemented in the amount of 200 mg/kg feed (group III) caused the vitamin E content of meat to increase significantly to 1.76 μg/g. In line with the above results, PUFA levels in group II were significantly higher than in group V.

There was no effect of the vitamin supplements, including β-carotene, on the level of vitamin A in meat. The vitamin A content was below the limit of quantitative determination of this compound. The absence of the effect of β-carotene conversion into retinol (vitamin A) may be attributed to the fact that it is not easily available from compound feeds (Handelman, 1996).

Highly significant differences were found in the content of crude fat depending on the gender of fatteners. The meat of gilts was characterized by a significantly lower content of crude fat compared with barrows, and by a lower content of dry matter, by 1.96 and 2.66%, 26.1 and 26.7%, respectively. It is supposed that the differences in the fat content of meat were related to the pigs' ability to deposit protein. In this respect, gilts are superior to barrows and thus their carcasses are characterized by lower fatness and higher meatiness (Fandrejewski, 1996).

Meat pH measured in gilts 24 h *post-mortem* was 5.44 and was significantly lower than that of barrows (5.55; $P<0.01$). The pH values found in gilts probably attest to their lower glycolytic potential compared with the barrows. Van Laack et al. (2001) indicated that, in pork, the ultimate pH (pHu) influence tenderness but its role has not been conclusively determined. In our experiment we could not determine whether this difference in pH_{24} between gilts and barrows causes the difference in tenderness of meat.

In the present study the best antioxidative effect on the *longissimus dorsi* muscle was observed in the group of fatteners receiving the combined supplement of β-carotene and vitamins C and E (group V).

CONCLUSIONS

It is concluded that the use of supplemental β-carotene, vitamins E and C in complete diets enriched in conjugated linoleic acid in the second period of fattening had no effect on the CLA level in meat. However, the tendency towards a higher CLA content in the meat of gilts was observed. Varied influence of antioxidants on the PUFA level was found. A higher level of UFA and PUFA and significantly lower SFA acid content was found in gilts compared with barrows. The experimental factors did not worsen the physicochemical characteristics of meat. The vitamin E content in meat increased when pigs received a higher dose. Supplemental β-carotene did not increase the level of vitamin A in meat. A tendency was observed towards a lower TBARS content after 3 months of frozen storage of meat in the group receiving a supplement of vitamins E and mixture of vitamins C, E and β-carotene. No significant interaction between gilts and barrows was observed according to the antioxidant used.

REFERENCES

AOAC, 1995. Official Methods of Analysis, Association of Official Analytical Chemists. 16th Edition. Arlington, VA

Banhegyi G., Braun L., Csala M., Puskas F., Mandl J., 1997. Ascorbate metabolism and its regulation in animals. Free Radical Biol. Med. 23, 793-803

Budsławski J., Drabent Z., 1972. Methods of Food Analysis (in Polish). WNT, Warszawa

CIE, 1976. CIE publication No. 15 (E-1.3.1), 1971/tc-1-1), Suppl. 2. Recommendations on uniform color spaces-color difference equations. Psychometric Color Terms, Com. Paris (France) Institute of de'Eclairage

Fandrejewski H., 1996. The importance of genotype in pig nutrition. In: Proceedings of the Seminar "Possibility of Genetic Improvement of the Pig Population in Poland for Economically Important Traits" (in Polish). National Research Institute of Animal Production (Editor). Balice (Poland), pp. 51-62

Folch J., Lees M., Stanley G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 266, 497-509

Grau R., Hamm R., 1953. Eine einfache Methode zur Bestimmung der Wasserbindung im Muskel. Naturwissenschaften 40, 29

Hanczakowska E., 2004. Effect of natural antioxidants in diets on fattening results and meat quality of fatteners (in Polish). Dissertation. National Research Institute of Animal Production (Editor). Balice (Poland), pp. 1-75

Handelaman G.J., van Kuijk F.J., Krinsky N.I., 1991. Characterization of products formed during the autooxidation of β-carotene. Free Radical Biol. Med. 10, 427-437

Hartmann S., Otten W., Kratzmair M., Seewald M.J., Iaizzo P.A., Eichinger H.M., 1997. Influences of breed, sex and susceptibility to malignant hyperthermia on lipid composition of skeletal muscle and adipose tissue in swine. Amer. J. Vet. Res. 58, 738-743

Hof K.H., West C.E., Westtrate J.A., Hautvast J.G., 1999. Dietary factors that affect the bioactivity of carotenoids. J. Nutr. 130, 503-506

Joo S.T., Lee J.I., Ha Y.L., Park G.B., 2002. Effects of dietary conjugated linoleic acid on fatty acid composition, lipid oxidation, color, and water-holding capacity of pork loin. J. Anim. Sci. 80, 108-112

Mersmann H.J., Houk J.M., Phinney G., Underwood M.C., 1973. Effect of diet and weaninig age on in vitro lipogenesis in young swine. J. Nutr. 103, 821-828

Monahan F.J., Gray J.I., Asghar A., Huag A., Shi B., Buckley D.J., Morrissey P., 1993. Effect of dietary lipid and vitamin E supplementation on free radical production and lipid oxidation in porcine muscle microsomal fractions. Food Chem. 46, 1-6

Müller H.L., Kirchgessner M., Roth F.X., Stangl G.I., 2000. Effect of conjugated linoleic acid on energy metabolism in growing-finishing pigs. J. Anim. Physiol. Anim. Nutr. 83, 85-94

Numa S., Nakanishi S., Hashimoto S., Iritani N., Okozaki T., 1970. Role of acetyl coenzyme A carboxylase in the control of fatty acid synthesis. Vitams. Horm. 28, 213-220

Nutrient Requirements of Pigs. Nutritive Value of Feedstuffs (in Polish), 1993. The Kielanowski Institute of Animal Physiology and Nutrition (Editor). Jabłonna (Poland)

Ohshima T., Fujita Y., Koizumi C., 1993. Oxidative stability of sardine and mackerel lipids with reference to synergism between phospholipids and tocopherol. J. Amer. Oil Chem. Soc. 70, 269-276

Ostrowska E., Cross R.F., Muralitharan M., Bauman D.E., Dunshea F.R., 2003. Dietary conjugated linoleic acid differentially alters fatty acid composition and increases conjugated linoleic content in porcine adipose tissue. Brit. J. Nutr. 90, 915-928

Packer L., 1991**.** Protective role of vitamin E in biological systems. Amer. J. Clin. Nutr. 53, 1050-1055

Palozza P., Krinsky N.I., 1991. The inhibition of radical-initiated peroxidation of microsomal lipids by both α-tocopherol and β-carotene. Free Radical Biol. Med. 11, 407-414

Pieszka M., Barowicz T., Migdał W., Pietras M., Migdał W., Kędzior W., 2004. Chemical composition and sensory traits of meat of fatteners fed with mixtures containing corn oil without or with the addition of α-tocopherol acetate. Pol. J. Food Nutr. Sci. 13/54, 65-69

Polish Standard PN-77/A-82058. Meat and Meat Products. Determination of pH

Salih M., Smith D.M., Price J.F., Dawson L.E., 1987. Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. Poultry Sci. 66, 1183-1188

Sawosz E., Chwalibog A., Niemiec T., Kosieradzka I., Skomiał J., 2005. Effect of nutrition on oxidative stress. J. Anim. Feed Sci. 14, Suppl. 1, 87-97

Ueda T., Igarashi O., 1987. Effect of coexisting fat on the extraction of tocopherols from tissues after saponification as pretreatment for HPLC determination. J. Micron. Analyt. 3, 15-25

Van Laack R.L.J.M., Stevens S.G., Stalder K.J., 2001. The influence of ultimate pH and intramuscular fat content on pork tenderness and tenderization. J. Anim. Sci. 79, 392-397

Zanardi E., Novelli E., Ghiretti G.P., Dorigoni V., Chizzolini R., 1999. Color stability and vitamin E content of fresh and processed pork. Food Chem. 67, 163-171