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Original scientific paper

FATTY ACID COMPOSITION OF PORK AS AFFECTED BY DIETARY VITAMIN E SUPPLEMENTATION AND COOKING

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The changes in the fatty acid composition of *m. Longissimus dorsi* and *m. Semimembranosus* in response to vitamin E supplementation in the diet (400 mg/kg feed) and cooking were studied in ♀Youna × ♂Pietrain pigs. Meat samples were cooked at 100 °C for 30 min. Fatty acid composition was determined and compared to that of non-processed samples. Feeding pigs with vitamin E had a significant effect on the increase in the content of C14:0 (myristic acid), C16:0 (palmitic acid) and C16:1 (palmitoleic acid) in *m. Longissimus dorsi* ($P < 0.01$), while in *Semimebranosus* muscle significant increase was detected only in C16:1 ($P < 0.05$). Regardless of cooking, the content of the total saturated (SFA) and polyunsaturated fatty acids (PUFA) remained unchanged in response to vitamin E in *Longissimus* and *Semimembranosus* muscles. Cooking of meat samples had a significant effect on the increase of the total saturated fatty acids content in the muscles, probably due to the high temperature. In terms of polyunsaturated fatty acids the effect of cooking was not consistent in *m. Longissimus dorsi*, while in *m. Semimembranosus* significant decrease ($P < 0.001$) in the content of C18:2 (linoleic acid), C20:3 (eicosantrienoic acid), C20:4 (arachidonic acid) and increase in C22:5 (docosanpentaenoic acid) was observed ($P < 0.001$).

Key words: vitamin E; pork; cooking; fatty acids

СОСТАВ НА МАСНИ КИСЕЛИНИ ВО СВИНСКО МЕСО ПОД ВЛИЈАНИЕ НА ВИТАМИН Е И НЕГОВА ТЕРМИЧКА ОБРАБОТКА

Беа проучувани промените во составот на масни киселини на *m. Longissimus dorsi* и *m. Semimembranosus* како одговор на диететскиот додаток (суплемент) витамин Е во исхраната (400 mg/kg храна) и термичката обработка на месото кај свињи ♀Youna × ♂Pietrain. Примероците месо термички се обработуваа на 100°C околу 30 минути. Составот на масни киселини беше утврден и спореден со необработените примероци. Исхраната на свињи со витамин Е имаше значително влијание врз зголемување на содржината на C14:0 (миристинска киселина), C16:0 (палмитинска киселина) и C16:1 (палмитолеинска киселина) во *m. Longissimus dors*i ($p < 0.01$), додека во мускулот *Semimebranosus* значително зголемување е откриено само во C16:1 ($P < 0.05$). И покрај термичката обработка, содржината на вкупно заситени (SFA) и полинезаситени масни киселини (PUFA) остана непроменета како одговор на витаминот Е во мускулите *Longissimus* и *Semimembranosus*. Термичката обработка на примероците месо имаше значително влијание врз порастот на вкупните заситени масни киселини во мускулите, што веројатно се должи на високата температура. Во однос на полинезаситените масни киселини, ефектот на готвењето не е постојан во *m. Longissimus dorsi*, додека во *m. Semimembranosus* има значително намалување ($p < 0.001$) во составот на C18:2 (линолна киселина), C20:3 (еикозантриенонска киселина) и C20:4 (арашидонската киселина), а беше забележано значително зголемување на C22:5 (докозанпентаенонска киселина), ($P < 0.001$).

Клучни зборови: витамин Е; свинско месо; термичка обработка; масни киселини

INTRODUCTION

Meat is most often consumed thermally treated. Various forms of thermal treatment exist and they alter more or less the sensory, nutritional and healthy quality of meat. Lipids are one of the main components to undergo changes due to high temperatures, including oxidative damage and transformation of their fatty acid composition. Oxidation is related to lipid characteristics and pro-oxidant to antioxidant ratio in meat, and recent studies focus on ways to increase the content of

natural antioxidant compounds (α-tocopherol or α-carotene) through feeding rather than use of synthetic ones *post mortem*. The positive effect of dietary vitamin E supplementation for increasing lipid oxidative stability of meat during pro-oxidative conditions has been reported in meat of ruminants [1, 2] and pork [3,4]. However relatively few research is done on the possible role of vitamin E for alteration of fatty acids in meat during thermal treatment [5, 6].

Therefore the aim of this work is to provide information on the effect of vitamin E in the diet of pigs on the fatty acids of meat and on their possible transformations during cooking process.

MATERIAL AND METHODS

Experimental animals

The experiment was carried out in the experimental farm of the Institute of Animal Science – Kostinbrod. Twenty ♀Youna × ♂Pietrain pigs were allocated in 2 groups (control and experimental) each containing 6 females and 4 castrated males. The pigs from both groups were fattened using concentrate (CP – 14.27%), as the diet of the experimental group was additionally supplemented with vitamin E in amounts of 400 mg/kg feed for a period of 10 weeks prior slaughter. The animals had *ad libitum* access to feed and water. The average weight of the pigs at the beginning of the experiment was 49.1 ± 5.71 kg and reached 94.75 ± 5.59 kg and 94.8 ± 4.54 kg at the end of the trial, respectively for the control and the vitamin E supplemented goup.

Slaughtering and sampling

The pigs were slaughtered in a standard slaughterhouse (at a distance of 5 km from the experimental farm). After slaughter the carcasses were kept for 24 h at 4°C. *Longissimus dorsi* and *Semimembranosus* muscles were dissected of each left side of the carcasses. Parts of the muscles were cut into small size pieces in parallel with the muscle fibers, put into glass tubes and boiled at 100°C for 30 minutes in water bath. Samples for determination of the fatty acid composition were taken from both raw and cooked meat.

Analysis of fatty acids

Fatty acid composition was analyzed in the Laboratory of Lipid Analysis in the Department of Ecology and Quality of Animal Production, Institute of Animal Science – Kostinbrod. Total lipids of the muscles were extracted according to the method of Bligh and Dyer [7]. Methyl esters of the lipids, isolated by preparative TLC were obtained using 0.01 % solution of sulphuric acid in dry methanol for 14 h, as described by Christie [8]. The fatty acid composition of total lipids was determined by GLC analysis using gas chromatograph C Si 200 equipped with capillary column (TR-FAME – 60 m × 0.25 mm × 0.25 μm) and hydrogen as a carrier gas. The oven temperature was first set at 160 °C for 0.2 min and then raised until 220 °C at a rate of 5 °C/min and hold for 5 minutes. The temperatures of the detector and injector were 230 °C. Methyl esters are identified comparing to the retention times of the standards. Fatty acids are presented as percentages of the total amount of the methyl esters identified [8].

Statistical evaluation

The results were statistically analyzed using two-ways ANOVA. The model included effects ascribed to vitamin E supplementation, cooking and their interaction on the fatty acid composition of *m. Longissimus dorsi* and *m. Semimembranosus*. The statistical evaluation was done using JMP v.7 software [9].

RESULTS AND DISCUSSION

Significant influence of the vitamin E supplementation ($P < 0.01$) was found on the contents of C14:0 (myristic acid), C16:0 (palmitic acid) and C16:1 (palmitoleic acid) in *m. Longissimus dorsi* (Table 1). The three fatty acids increased in the animals receiving vitamin E in both raw and cooked samples. The content of C18:0 (stearic acid) and C18:1 (oleic acid) remained unchanged in response to vitamin E in the diet.

Although no significant influence on the contents of the individual polyunsaturated fatty acids (PUFA) due to the vitamin E was observed, they tended to decrease in the non-thermally treated samples of the pigs from the vitamin E supplemented group. In a study with the same animals we found more pronounced effect of diet leading to decrease in PUFA in non-processed, stored up to 6 months at –20 °C muscles in pigs that received vitamin E (unpublished data). The above mentioned tendency towards decreased content was particularly observed for C18:2 (linoleic acid) and C20:4 (arachidonic acid).

The content of the major saturated acids (C16:0, C18:0) in *m. Semimembranosus* (Table 1) was not changed in response to the vitamin E in the diet of the pigs. The content of C17:0 (margaric acid) was lower ($P < 0.01$) in the vitamin E supplemented group, while C16:1 displayed higher contents in both raw and cooked samples ($P < 0.05$). The content of C20:3 (eicosantrienoic acid) was also higher in the animals receiving vitamin E ($P < 0.05$).

No significant difference in response to the dietary vitamin E supplementation was observed in the total amounts of saturated fatty acids (SFA) as well as PUFA in *Longissimus* and *Semimembranosus* (Table 2) muscles. Only the content of n-3 PUFA differed significantly ($P < 0.05$) showing lower content in samples of *m. Semimembranosus* of the supplemented pigs.

Studies report different influence of vitamin E in the diet on the fatty acid composition in meat that could be due to the levels of dietary supplement as well as the duration of the experimental period. In studies with cattle Shorland *et al.* [10] showed significant reduction in the content of the hypercholesterolemic C12:0, C14:0 and C16:0 and increase of C18:0 when feeding the animals with 500 mg/kg vitamin E in the diet. Onibi *et al.* [11] observed decreased content of the saturated fatty acids in pork when fed 200 mg/kg vitamin E, which is not in line with our results. However they reported also decreased content of polyunsaturated fatty acids. In broilers after cooking, Skřivanová *et al.* [5] found decrease in the content of C20:5 (eicosanpentaenoic acid), C22:5 (docosanpentaenoic acid) and C22:6 (docosanhexaenoic acid) when supplemented diet with vitamin E in amounts of 100g/kg and copper.

Thermal treatment of *m. Longissimus dorsi* samples had strong influence on the contents of the major saturated fatty acids – C16:0 and C18:0 ($P < 0.001$) that was not dependent on the presence of vitamin E in the diet. As a result of cooking the content of C16:0 and C18:0 increased in both control and supplemented animals. The contents of the monounsaturated C16:1 and C18:1 remained unaltered after cooking, although tendencies towards decreased content of C16:1 were observed.

Table 1

Influence of the dietary vitamin E supplementation and cooking on the fatty acid composition of m. Longissimus dorsi and m. Semimembranosus in pigs (values presented as least squares means)

Fatty acids, %	*m. Longissimus dorsi*				S.E.	Significance			*m.Semimembranosus*				S.E.	Significance		
	Control		Vitamin E			Group	Cooking	Interaction	Control		Vitamin E			Group	Cooking	Interaction
	Raw	Cooked	Raw	Cooked					Raw	Cooked	Raw	Cooked				
C14:0[1]	1.22	1.14	1.43	1.36	0.20	**	NS	NS	0.96	1.22	1.08	1.24	0.19	NS	**	NS
C16:0[2]	22.71	24.43	23.62	25.27	0.91	**	***	NS	22.38	24.54	22.68	24.36	0.96	NS	***	NS
C16:1[3]	2.56	2.43	3.02	2.91	0.45	**	NS	NS	2.49	2.72	2.79	3.09	0.40	*	**	NS
C17:0[4]	0.19	0.31	0.19	0.23	0.06	NS	***	NS	0.24	0.32	0.20	0.26	0.04	**	***	NS
C18:0[5]	10.98	12.89	10.55	12.76	1.02	NS	***	NS	11.07	12.54	10.95	12.36	1.01	NS	***	NS
C18:1[6]	46.92	47.93	47.64	46.11	2.11	NS	NS	NS	45.90	45.73	45.32	46.32	2.72	NS	NS	NS
C18:2[7]	10.78	8.92	9.55	8.86	1.29	NS	***	NS	12.02	10.51	12.18	9.56	1.76	NS	***	NS
C18:3n-6[8]	0.13	0.12	0.12	0.11	0.03	NS	NS	NS	0.13	0.14	0.12	0.14	0.04	NS	NS	NS
C18:3n-3[9]	0.24	0.26	0.23	0.29	0.04	NS	**	NS	0.28	0.28	0.28	0.28	0.03	NS	NS	NS
C20:2[10]	0.34	0.37	0.31	0.35	0.06	NS	*	NS	0.40	0.34	0.39	0.30	0.06	NS	NS	NS
C20:3[11]	0.26	0.13	0.24	0.16	0.04	NS	***	NS	0.26	0.14	0.28	0.17	0.04	*	***	NS
C20:4[12]	3.33	0.96	2.80	1.40	0.48	NS	***	**	3.56	1.27	3.54	1.73	0.71	NS	***	NS
C20:5[13]	0.08	0.02	0.08	0.04	0.04	NS	**	NS	0.08	0.01	0.08	0.06	0.02	**	***	**
C22:5[14]	0.25	0.08	0.22	0.14	0.04	NS	***	**	0.21	0.24	0.10	0.13	0.02	NS	***	NS

Significance effects:* $P < 0.01$; ** $P < 0.01$; *** $P < 0.001$; NS – non significant;

[1]C14:0 – myristic acid; [2]C16:0 – palmitic acid; [3]C16:1 – palmitoleic acid; [4]C17:0 – margaric acid; [5]C18:0 – stearic acid; [6]C18:1 – oleic acid; [7]C18:2 – linoleic acid; [8]C18:3 n-6-γ-linolenic acid; [9]C18:3 n-3-α-linolenic acid; [10]C20:2 – eicosandienoic acid; [11]C20:3 – eicosantrienoic acid;[12]C20:4 – arachidonic acid; [13]C20:5 – eicosanpentaenoic acid; [14]C22:5 – docosanpentaenoic acid.

Table 2

Influence of the dietary vitamin E supplementation and cooking on the total amounts of fatty acids in m. Longissimus dorsi and m. Semimembranosus in pigs (values presented as least squares means)

Fatty acids, %	*m.Longissimus dorsi*				S.E.	Significance			*m. Semimembranosus*				S.E.	Significance		
	Control		Vitamin E			Group	Cooking	Inter-action	Control		Vitamin E			Group	Cooking	Inter-action
	Raw	Cooked	Raw	Cooked					Raw	Cooked	Raw	Cooked				
SFA[1]	35.10	38.77	35.78	39.62	1.50	NS	***	NS	34.65	38.62	34.91	38.22	1.65	NS	***	NS
PUFA[2]	15.41	10.86	13.55	11.35	1.82	NS	***	*	16.94	12.93	16.97	12.37	2.49	NS	***	NS
n-6[3]	14.84	10.50	13.02	10.88	1.76	NS	NS	*	16.37	12.41	16.51	11.90	2.43	NS	***	NS
n-3[4]	0.57	0.36	0.53	0.47	0.08	NS	***	*	0.57	0.53	0.46	0.47	0.08	*	***	NS
PUFA/SFA	0.44	0.28	0.38	0.28	0.05	NS	***	NS	0.49	0.33	0.49	0.32	0.07	NS	***	NS
AI[5]	0.42	0.45	0.47	0.51	0.02	**	***	NS	0.40	0.48	0.41	0.47	0.02	NS	***	NS
TI[6]	1.03	1.22	1.06	1.25	0.1	NS	***	NS	1.00	1.19	1.07	1.18	0.07	NS	***	NS

Significance effects:*P < 0.01; **P < 0.01; ***P < 0.001; NS – non significant
[1]SFA – total amount of the saturated fatty acids
[2]PUFA – Total amount of the polyunsaturated fatty acids
[3]n-6 – ΣC18:2, C18:3n-6, C20:2, C20:3, C20:4
[4]n-3 – ΣC18:3n-3, C20:5, C22:5
[5]AI – Atherogenic index = [(4×C14:0)+C16:0)]/(MUFA+PUFA)
[6]TI – Thrombogenic index = (C14:0+C16:0+C18:0)/(0.5×MUFA+0.5×n-6PUFA+3×n-3PUFA+n-3PUFA/n-6PUFA)

In terms of the polyunsaturated fatty acids the influence of the cooking was not consistent in *m. Longissimus dorsi.* Significant decrease due to high temperature was observed in the content of linoleic acid ($P < 0.001$), eicosantrienoic acid ($P < 0.001$) and eicosanpentaenoic acid ($P < 0.01$) in control and supplemented animals. On the other hand increase in the contents of C18:3n-3(α-linolenic acid) ($P < 0.01$) and C20:2 (eicosandienoic acid) ($P < 0.05$) was determined for both groups in the cooked samples. Decrease in the content of C20:4 and C22:5 was observed in the cooked muscle in control and supplemented animals, however significant interaction between dietary vitamin E supplementation and cooking was observed ($P < 0.01$). It was confirmed by the higher contents of these two fatty acids in the cooked samples of supplemented pigs when comparing to the cooked samples of the control group. The same was observed for the total amount of PUFA, n-6 and n-3 where significant interaction diet × cooking was determined ($P < 0.05$).

As in *Longissimus* muscle the cooking affected considerably the fatty acid composition in *m. Semimembranosus.* It led to significant increase of the content of C14:0 ($P < 0.01$), C16:0, C17:0, C18:0 ($P < 0.001$) as well as C16:1 ($P < 0.01$). The contents of C18:2, C20:3 and C20:4 were significantly lower ($P < 0.001$) in the cooked samples of both control and supplemented animals while the content of C22:5 became higher ($P < 0.001$).

These results indicate different response of the two muscles to the cooking in regards to their fatty acid composition. The higher content of C14:0, C16:0 and C18:0 correspond to the higher content of lipids in the cooked meat. Since the first two fatty acids have been associated with increased risk of cardiovascular diseases it could be said that their transformation in the conditions of this experiment is not positive in terms of health. The influence of the cooking concerning the total amounts of SFA, PUFA, n-6 and n-3 confirmed the already observed for the individual fatty acids. In general the content of SFA increased ($P < 0.001$) while those of PUFA, n-6 and n-3 decreased due to cooking independently of the dietary vitamin E supplementation. Thermal treatment has been reported to have different effect on the fatty acid composition of meat. Smith *et al.* [12] and Harris *et al.* [13] did not detect any changes in the fatty acid profile in sirloin after cooking. Our results are in agreement with those of Duckett and Wagner [14] who observed increase in stearic acid in total lipids of cooked beef while the contents of linoleic, lino-

lenic and arachidonic acid decreased. Souza *et al.* [6] also reported significant increase in SFA between raw *Biceps femoris* and cooked ham, made of the same muscle. However they observed increased PUFA and MUFA (monounsaturated fatty acids) between the raw and cooked meat. Skřivanová *et al.* [5] reported decreased saturated fatty acids and increased MUFA in broiler meat after stewing and roasting which is contrary to our findings. These differences could be implicated to the method of thermal treatment as well as duration.

PUFA/SFA ratio and two distinct indices – atherogenic index (AI) and thrombogenic index (TI), were proposed as indicators for the risk of cardiovascular diseases in humans. In both muscles the atherogenic and thrombogenic indices are calculated according Ulbricht and Southgate [15]. The ratio PUFA/SFA in raw muscle samples varied between 0.44 and 0.38 in *m. Longissimus dorsi* and 0.49 in *m. Semimembranosus*. According to the nutritional recommendations of WHO [16] the values of this ratio should be >0.4. However in the cooked samples of both muscles significant decrease of this ratio was observed.

Atherogenic and thrombogenic indices increased in thermal treated samples in *m. Longissimus dorsi* and *m. Semimembranosus* ($P < 0.001$) mainly due to the content of SFA that increase in both groups after cooking. However except in *m. Longissimus dorsi* of the vitamin E supplemented group, the values of AI remain below 0.5 that is recommended by Ulbricht and Southgate [15].

CONCLUSIONS

Dietary vitamin E supplementation affected significantly the content of C14:0 (myristic acid), C16:0 (palmitic acid) and C16:1(palmitoleic acid) in *m. Longissimus dorsi* showing higher values in the vitamin E supplemented animals, while in *m. Semimembranosus* significant increase was observed for C16:1 (palmitoleic acid) in the experimental animals in raw and cooked meat samples. The content of the total saturated and polyunsaturated fatty acids remained unchanged in response to vitamin E in *Longissimus* and *Semimembranosus* muscles, regardless of the thermal treatment. Strong influence of cooking was observed on the fatty acid profile in meat. As a result of high temperature the content of the saturated fatty acids increased considerably. In terms of PUFA the influence of cooking was not consistent in *Longissimus* muscle while in *m. Semimembranosus* significant decrease in the content of C18:2 (linoleic acid), C20:3 (eicosatrienoic acid), C20:4 (arachidonic acid) and increase in C22:5 (docosapentaenoic acid) was observed.

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