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Original research paper

HEAT TREATMENT EFFECT ON FATTY ACID COMPOSITION IN DIFFERENT PIG TISSUES

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ABSTRACT: Linseed is one of the most useful crops that has been cultivated as a commercial plant all over the world. Recently there has been a growing interest in linseed oil due to the high concentration of linoleic and especially α-linolenic acid. Since animals are not able to synthesize these essential fatty acids, changes in fatty acid content in meat can be achieved through the changes in animal diet. The aim of this study was to evaluate the influence of diet supplemented with linseed rich additive on fatty acid profile and omega fatty acids content in pig meat. Furthermore, fatty acid composition of roasted meat of pigs fed with control and experimental diet was investigated.
Twelve pigs were divided in a control and experimental group and grown to 110 kg of live weight. The experimental group was fed a standard diet enriched with 2.5% of commercial additive. Fatty acid composition of fresh meat samples and heat treated meat were determined by GC-FID. Meat was roasted in oven at the temperature of 80 - 85^{0}C until the temperature in the centre reached 69^{0}C (about 1 hour). STATISTICA software, version 10 was used for performing ANOVA and Fishers comparison of means.
Samples from the experimental group fed with linseed enriched diet showed significantly higher ($p < 0.01$ and $p = 0.01$, respectively) content of both omega 3 (n-3) (8.25% vs. 1.33%) and omega-6 (n-6) fatty acids (25.68% vs. 20.68%) in all tissues, thus making it better for a human consumption from a health perspective. Heat treatment significantly decreased ($p < 0.05$) the content of stearic and linoleic acid in control tissue samples, but decrease was insignificant ($p > 0.05$) in samples from the experimental group.
In conclusion, diet enriched with linseed had a beneficial effect on the majority of monitored parameters in the study.

Key words: *linseed diet, essential fatty acids, pig meat, heat treatment*

INTRODUCTION

Dietary fats, especially essential fatty acids, which cannot be endogenously synthesized by humans and other mammals, play an important role in human health and well being (Beare-Rogers *et al.*, 2001). Many clinical, epidemiological and biological studies ascribe a particular significance to n-3 polyunsaturated fatty acids (PUFA). Results from those studies confirmed their anti-inflammatory, antithrombogenic, and hypotriglyceridemic properties; they are also active against some types of cancer like colon, breast and prostate cancer (Rose *et al.*, 1999; Connor, 2000). However, these beneficial effects are limited by the fact that the modern western diet is rather low in n-3 fatty acids, while it is very high in n-6 fatty acids content, and it is well known fact that increased levels of n-6 fatty acids are associated with an increase in chronic diseases (Givens *et al.*, 2006; Enser *et al.*, 2000).
The significance of omega-3 fatty acids in human nutrition is presented. As essential substances they cannot be synthesized in the organism, but have to be introduced through

diet. Also, the significance of some essential n-6 fatty acids as well as their mutual relation, are presented. The role of n-3 fatty acids in animal nutrition is also pointed out in this paper, introduced or consumed by animals either by grazing or as diet supplement, which influence improvement of their production, reproduction and health performances (Sretenović et al., 2009).

The main reason for this is production of animal feed from grains rich in n-6 fatty acids which subsequently leads to meat rich in the same type of fatty acids (*Crawford, 1968*). Being aware of n-3 PUFA benefits and health promoting effects, the nutritionists recommend a diet rich in n-3 fatty acids as well as a lower n-6/n-3 ratio from the current 15-20:1 to 1-4:1 (Simopoulos, 2002; Okanović et al., 2010).

Since animal diet determines the fatty acid composition in meat, changes in fatty acids ratio and composition in meat can be achieved through the changes in animal diet (Mourot and Hermier, 2001; Ivanov et al., 2009). Most common way to perform this is by enriching the animal feed with fish oil/fish meals or with plant oils rich in n-3 PUFA or seed meals such as linseed (Raes *et al.*, 2004; Kouba *et al.*, 2003). Linseed, containing about 40% of oil, has long been used in human and animal diet. Oil from the seeds contains remarkably high content of linoleic (LA, 18:2, n-6) and especially α-linolenic acid (ALA, 18:3, n-3). Furthermore, linseed is the richest oilseed source of ALA (Juárez *et al*., 2010, Łukaszewicz *et al*., 2004, Ivanov *et al.*, 2012). Monogastric animals (like pigs) are better target for this approach, because the dietary fatty acids are absorbed from the intestine unchanged (Enser *et al.*, 2000). In this way, composition of fatty acids in meat could be modified by dietary means ultimately improving nutritional and health value of the meat (Stevanović et al., 2011).

The objective of presented study was to investigate the influence of linseed enriched diet (rich in n-3 PUFA) on fatty acid composition of pig meat. This was done by supplementing the standard, commercial feed with linseed and observing the effects of such feeding (with standard diet used as a control) on changes in fatty acid composition of various pig tissues. Furthermore, the samples were roasted in order to obtain information about fatty acid degradation caused by the heat treatment.

MATERIAL AND METHODS

Animals and diet

The total twelve pigs, Pietrain x (Landrace x Great Yorkshire) were used in the study, which was conducted at the pig farm „Sabo Janos", Jermenovci, Serbia.

Twelve pigs were divided into two groups and fed with two types of diet, a standard diet and diet enriched with 2.5% of Vitalan® (Vitalac, France), until reaching approximate 110 kg of live weight, when they were slaughtered. Vitalan® contains 85% extruded linseed, which made the diet rich in n-3 acids and the rest were wheat bran and antioxidants. The composition of the diets is shown in Table 1.

Table 1: Composition of diets for pigs

Components (%)	Control group	Experimental group
Vitalan®	0	2.5
Maize	51.0	50.0
Barley	28.0	26.8
Soybean meal	18.0	17.7
Premix	2.5	2.5

Acidifier	0.5	0.5
Total	100.0	100.0

Slaughtering and sampling

The animals were slaughtered and samples of pig meat (*M. Longissimus dorsi,* bacon and back fat), 3 pieces (200 g each) from both groups were collected and kept in the refrigerator at 4^0C. A half amount of every sample was roasted in the oven at the temperature of 80 - 85^0C until the temperature in the centre of the meat reached 69^0C (about 1 hour). After 24 hours, the samples were sent to the laboratories of Institute of Food Technology in Novi Sad, where fatty acid analysis and sensory evaluation were performed. Each sample was coded with a letter: C for samples from control group and E for samples from experimental group.

Fat extraction for fatty acid analysis

Supercritical fluid extraction with CO_2 was used for preparation of fat extracts, as recommended for fatty acid analysis (Ivanov *et al.*, 2012). Extractions were performed on a LECO TFA2000 fat analyzer with the method adopted from existing LECO procedures (Leco corporation, 2003). Infusorial soil (flux calcined infusorial soil, up to 54% crystalline silica, cristobalite < 50%, quartz < 4%, produced by LECO Corporation, MI, USA) was used as absorbent to remove traces of water from samples. The preselected meat samples were homogenized with food processor. 1.0 g of homogenized meat was vigorously mixed with 2.2 g of absorbent and this way prepared mixture was transferred into a metal extraction thimble (12 cm length and 10 mm diameter) for the extraction. After finishing the extraction step, the instrument was depressurized, and extracts in collection vials were de-gassed for ten minutes, until achieving constant weight of extracts.

Fatty acid determination

From the extracted lipids, fatty acid methyl esters were prepared with method that uses boron trifluoride/methanol solution (Veresbaranji, 1996). Nitrogen gas was used for drying and removing solvents from fatty acid methyl esters. Obtained samples were analyzed by a GC Agilent 7890A system with FID, auto-injection mode, equipped with fused silica capillary column (DB-WAX 30 m, 0.25 mm, 0.50 µm). Helium was used as a carrier gas (purity > 99.9997 vol. %, flow rate = 1.26 ml/min). The fatty acids peaks were identified by comparison of retention times with retention times of standards from Supelco 37 component fatty acid methyl ester mix (Cat. No. 47885-U, Supelco, PA, USA) and with data from internal data library, based on previous experiments and fatty acid methyl ester determination on GC-MS. Results were expressed as mass of fatty acid or fatty acid group (g) per 100 g of fatty acids, and as a ratio between n-6 and n-3 fatty acids.

Soxhlet extraction – free fat determination

Soxhlet extractions were performed with a Büchi 810 Soxhlet fat extraction apparatus (Soxtec system HT, 1043 Extraction Unit, Foss Tecator AB, Höganäs, Sweden) in accordance with manufacturer procedure and AOCS Method Ba 3-38 (AOCS, 2001). Extractions were performed with petroleum ether (40 - 60º) solvent. Sample size was 3g and extraction time was 1.5h, at 80 ºC. After removal from the apparatus, extracted lipids were allowed to cool at room temperature while passing air over the samples for 1 minute, and then dried in desiccant pouch until they reached constant weight (approximately 60 minutes). Fat content was expressed as the percentage, by weight (gravimetrical method).

Statistical analysis

STATISTICA software version 9 (Statsoft, Tulsa, OK, USA) was used for analyzing variations (analysis of variance – ANOVA), and for Fishers LSD comparison of means and pairwise comparison. Differences among means with probability $p \leq 0.05$ were accepted as representing statistically significant differences, and differences among means with $0.05 \leq p \leq 0.10$ were accepted as representing tendencies to differences.

RESULTS AND DISCUSSION

Effects of the diets used in the experiment on composition of fatty acid group and total fat content of pig tissues are presented in Table 2. Samples of *M. longissimus dorsi* of the control and experimental groups showed significant differences in fatty acid composition. SFA content decreased significantly ($p = 0.006$), monounsaturated fatty acids (MUFA) content also significantly ($p = 0.04$) decreased, and PUFA and unsaturated fatty acids (UFA) contents significantly ($p = 0.03$ and $p = 0.02$, respectively) increased in experimental groups. Experimental diet had less influence on back fat fatty acid composition, but increase in n-3 fatty acid content was still significant ($p < 0.05$).

Linseed diet positively affected n-6/n-3 ratio of all samples by decreasing it below the desirable level. Being aware of n-3 PUFA benefits and health promoting effects, the nutritionists recommend a diet rich in n-3 fatty acids, as well as a lower n-6/n-3 ratio from the currently common 15-20:1 to 1-4:1 (Gebauer et al., 2006, Raes *et al.*, 2004).

Table 2. Diet effects on composition of fatty acid group and total fat content of pigs tissues

		Fatty acids (% of total lipids)					*n-6/n-3*	UI*	Total fat
		SFA	MUFA	PUFA	*n-6*	*n-3*	ratio		content (%)
Meat	C	17.28 ± 0.47	59.63 ± 0.30	23.09 ± 0.31	19.61 ± 0.26	2.49 ± 0.17	7.88	1.09	5.11 ± 0.08
	E	13.30 ± 0.38	52.17 ± 0.35	36.44 ± 0.33	24.22 ± 0.06	10.89 ± 0.35	2.22	1.37	5.57 ± 0.06
	Probability	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$			
Back fat	C	17.95 ± 0.40	57.10 ± 0.26	23.95 ± 0.34	22.36 ± 0.16	0.71 ± 0.38	31.49	1.07	86.50 ± 0.01
	E	17.73 ± 0.43	56.61 ± 0.20	24.31 ± 0.23	27.20 ± 0.17	7.20 ± 0.16	3.78	1.34	82.90 ± 0.03
	Probability	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p < 0.05$	$p < 0.05$			
Bacon	C	16.98 ± 0.35	61.14 ± 0.16	21.59 ± 0.14	20.07 ± 0.13	0.79 ± 0.12	25.41	1.05	28.10 ± 0.03
	E	14.69 ± 0.30	51.23 ± 0.18	35.87 ± 0.16	26.11 ± 0.14	6.67 ± 0.16	3.91	1.25	26.10 ± 0.04
	Probability	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$			

The results are presented as mean ± SD

*UI – Unsaturation index = average number of double bonds per fatty acid residue

It is known that UFA containing double bonds are easily oxidized, and thus fatty acid composition can influence the palatability of meat, as well as that the effect of fatty acids on meat tenderness is due to the different melting points of individual fatty acids, especially stearic and linoleic acid (Jeong *et al.*, 2010). Changes in the content of these two acids in *M. longissimus dorsi* are shown in Table 3, as a result of the differences in composition of standard (control) and linseed enriched diet used for feeding of pigs.

Table 3. Changes in content of stearic, linoleic and omega fatty acids in pigs *M. longissimus dorsi* muscle after roasting

		Fatty acids (% ot total lipids)			
		Stearic acid	Linoleic acid	*n-6*	*n-3*
Control diet	Fresh	7.33 ± 0.19	19.96 ± 0.09	19.96 ± 0.09	2.49 ± 0.17
	Roasted	6.83 ± 0.40	19.61 ± 0.26	19.61 ± 0.26	0.69 ± 0.25
	Mean difference	0.5	0.35	0.35	1.8
	Probability	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
Experimental diet	Fresh	4.94 ± 0.17	24.22 ± 0.06	24.53 ± 0.12	10.89 ± 0.35
	Roasted	4.85 ± 0.19	24.53 ± 0.12	24.22 ± 0.06	7.21 ± 0.31
	Mean difference	0.09	0.31	0.31	3.68
	Probability	$p > 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

The results are presented as mean ± SD

After heat treatment, the contents of both fatty acids were lower in control, as well as in experimental samples. The differences were significant ($p < 0.05$) in all cases, except for the stearic acid in samples of meat of pigs fed with experimental diet. Linseed enriched diet positively influenced the content of linoleic acid, and therefore the tenderness of *M. longissimus dorsi* muscle. Previous experiments reported that meat from piglets fed with diet enriched in linseed by adding commercial additives had pleasant color and juicy taste (Okanović *et al.*, 2011).

Roasting of meat caused increase in n-6/n-3 as shown in Fig. 1, thus making it less desirable and suitable for human consumption.

Positive effect of linseed diet is reflected in the fact that increase of n-6/n-3 ratio was statistically significant ($p < 0.01$) only for meat samples of pigs fed with control diet. The content of n-3 fatty acids in control samples was notably low even before roasting, and the heat treatment decreased it below the content of 1%.

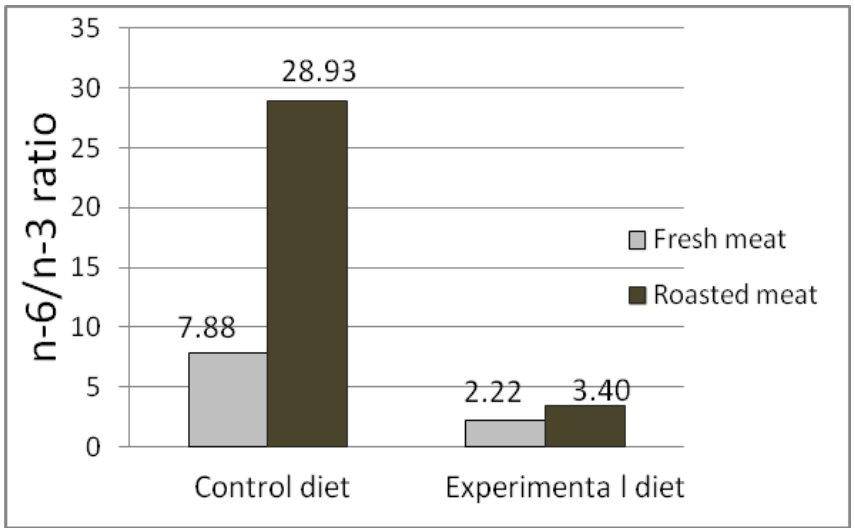

Fig. 1. n-6/ n-3 ration in *M. longissimus dorsi* muscle before and after roasting

CONCLUSION

Presented results have proven that addition of linseed in diet for pigs has multiple benefits considering healthy, nutritional and sensory characteristics of pig meat. Therefore, use of linseed enriched diet in pig feed can be recommended for obtaining meat with desirable fatty acid composition. Enhanced fatty acid composition of fresh meat provides better quality of heat treated meat from the lipid nutrition standpoint.

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УТИЦАЈ ТЕРМИЧКОГ ТРЕТМАНА НА МАСНОКИСЕЛИНСКИ САСТАВ СВИЊСКОГ МЕСА

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Сажетак: Лан представља једну од најкориснијих биљних култура, која се комерцијално гаји широм света. У последње време интерес за лањеним уљем нагло је порастао, пре свега због високог садржаја линолне, а нарочито α-линоленске киселине. С обзиром да сисари нису у стању да синтетишу ове есенцијалне масне киселине, промена састава масних киселина у месу животиња постиже се кроз измењен начин исхране животиња. Циљ овог истраживања био је да се одреди маснокиселински профил свињског меса, са акцентом на омега-масне киселине. Поред тога, испитан је и састав масних киселина печеног меса свиња храњених контролном и експерименталном смешом.

Дванаест јединки подељено је у контролну и експерименталну групу и узгајано до постигнуте масе од 110 kg живе ваге. Експериментална група је храњена стандарднм смешом уз додатак 2,5% комерцијалног адитива. Састави масних киселина узорака свежег и печеног меса одређени су помоћу ГЦ-ФИД. Месо је печено у пећници на температури 80 – 85 ^{0}C све док се није постигла температура од 69^{0}C у центру узорка (око сат времена). STATISTICA софтвер употребљен је за извођење анализе варијансе и Фишеровог поређења средњих вредости.

Узорци из експерименталне групе имали су значајно виши садржај омега-3 (8.25% наспрам 1.33%) и омега-6 масних киселина (25.68% наспрам 20.68%) у свим ткивима ($p < 0.01$ и $p = 0.01$, редом), чинећи их погоднијим за људску исхрану са здравственог аспекта. Термички третман значајно је снизио ($p < 0.05$) садржај стеаринске и линолне киселине у контролним узорцима, али снижење садржаја ове две масне киселине није било статистички значајно ($p > 0.05$) у узорцима из експерименталне групе.

На крају је закључено да лањено семе има благотворно дејство на већину праћених параметара у овом испитивању.

Кључне речи: *исхрана на бази лањеног семена, есенцијалне масне киселине, свињско мео, термички третман*

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