Indian Journal of Animal Sciences **90** (3): 446–450, March 2020/Article

Meat quality and fatty acid profile in *M. longissimus lumborum et thoracis* in Prestice Black-Pied pigs fed with linseed diet

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Received: 4 June 2019; Accepted: 20 August 2019

ABSTRACT

The study was designed to evaluate the effect of diet with supplementation of linseed scrap fed to Prestice Black-Pied pigs on carcass parameters, meat quality and oxidative stability. We also evaluated the effect of the diet on fatty acid profile in *M. longissimus lumborum et thoracis*. Barrows (30) of an indigenous breed Prestice Black-Pied pig were included in the experiment. The pigs fed with the linseed diet (L) were characterized by intramuscular fat lower by 1.1% in comparison with the control group (C). Of the saturated fatty acids, C6:0, C8:0 increased while C16:0, C17:0, C18:0 decreased in the experimental group of pigs fed with the L diet when compared to pigs of the C group. Of the monounsaturated fatty acids, a significant decrease of C18:1 n-9 was recorded in the experimental group. The most significant differences were found in polyunsaturated fatty acids (PUFA), where the L group showed an increase in C18:3 n-3, C20:3 n-3, C20:4 n-3 and also C20:5 n-3 a C22:5 n-3. These findings corresponded to higher content of n-3 PUFA and more favourable n-6/n-3 PUFA ratio in the meat of L group against the C group. A higher PUFA/SFA ratio was recorded in the experimental group.

Keywords: Fatty acid, Indigenous breed, Linseed, Meat quality parameters, Prestice Black-Pied

Representation of fatty acids in human nutrition is an important issue from the medical point of view. High content of saturated fatty acids in human diet can be an important factor in development of cardiovascular diseases (Wood *et al.* 2003). Pork meat is rich in fatty acids that are in unfavourable n-6/n-3 PUFA ratio, which is significantly influenced by the way pigs are fed, with utilization of plants rich in linoleic acid (Tartrakoon *et al.* 2016). There have been many studies focused on improvement of n-6/n-3 ratio in pork meat, since pork can be a cheap source of polyunsaturated fatty acids (PUFA). Saturated and unsaturated fatty acids are synthesized *in vivo* in pig body, but some of them cannot be synthesized within organism, such as linoleic and linolenic acid, and these are received in feeds (Wood *et al.* 2003, Ruxton *et al.* 2005, Vaclavkova *et al.* 2016). According to Rentfrow *et al.* (2003), linseed is a feed that causes increase of n-3 PUFA in pork meat and improves the n-6/n-3 PUFA ratio. Huang *et al.* (2008) used a mixture containing 10% of linseed for feeding of fattening pigs 30, 60 and 90 days before slaughter. The content of n-3 PUFA in *M. longissimus dorsi* and backfat increased linearly with the length of feeding with the experimental mixture, the concentration of α-linolenic acid increased from 0.49% to 4.15%. Guillevic *et al.* (2009) conducted a study on barrows and evaluated the effect of linseed diet with high content of α-linolenic acid and diet with supplementation of sunflower scrap and a linoleic acid rich oil on fatty acid profile of fat and muscle tissue in pigs. The content of n-3 PUFA increased in pigs fed with mixture supplemented with linseed.

Kralik *et al.* (2010) emphasize that content of fatty acids depends on factors other than diet itself. It can be age, sex, sensitivity to stress, breeding method, breed or genotype of pigs. Lorenzo *et al*. (2012) reported more favourable n-3/n-6 ratio in a local Spanish breed Celta as well as Kasprzyk *et al.* (2015) in a Polish breed Pulawska, which suggests a certain potential of these local breeds. Prestice Black-Pied pig represents an indigenous Czech breed from the western region of the Czech Republic. Since 1992 it has been listed in the National program of genetic resources under coordination of the European Regional Focal Point for Farm Animal Genetic Resources (Matoušek *et al.* 2016, Nevrkla *et al.* 2017).

The study was designed to evaluate effects of diet supplemented with linseed scrap fed to Prestice Black-Pied pigs on carcass parameters value, and meat quality and oxidative stability. We also evaluated effect of this diet on fatty acid profile in *M. longissimus lumborum et thoracis.*

MATERIALS AND METHODS

Animals, diets and housing: Barrows (30) native breed Prestice Black-Pied pig were included in the experiment. The barrows were divided into 2 groups (15 animals per group) fed with 2 different feed mixtures, viz. control feed

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mixture (C) and experimental feed mixture (L) with addition of the linseed scrap (7%). Composition of the feed mixtures, viz. nutrient contents and fatty acid groups are shown in Table 1. Animals of the same age and weight were divided randomly into 2 groups. The barrows were included in the experiment at the age of 138 days. The average weight of animals in the control was 68.11±4.98 kg and in the experimental group 68.93±5.02 kg and they were slaughtered when 206 days old weighing 114.82±7.52 kg in the control and 115.68±6.98 kg in the experimental group. For the duration of the experiment, the feed and water was available for the pigs *ad lib*. Mean daily feed mixture consumption (total for each) was 2.23 kg/animal/day in the control and 2.13 kg/animal/day in the experimental group. Feed conversion was 3.25 kg in the control and 3.10 kg in the experimental group. The barrows were housed in an indoor experimental stable in 2 pens of 15 animals. Part of the floor was concrete and part was slatted. The fence was made of plastic in combination with galvanized profiles. Each pen was equipped with three drinkers and a self-filling feeder.

Table 1. Components, nutrient composition and fatty acid groups in feeding mixtures

Parameter	Control (C)	Linseed scrap (L)
Ingredients (*g/kg*)		
Wheat	634	564
Barley	120	120
Soybean meal, extracted	80	80
Linseed	0	70
Rapeseed meal	70	70
Wheat bran	30	30
Malt sprouts	30	30
Rapeseed oil	2	2
Limestone	14.5	14.5
Salt	4	4
Monocalcium phosphate	4.5	4.5
Magnesium oxide	1	1
Amino acids and vitamins[1]	10	10
Nutrients (*g/kg*)		
Dry matter	895.92	896.03
Starch	439.60	389.78
Crude protein	162.20	173.27
Ash	50.70	54.21
Fat	24.98	40.40
Crude fibre	37.69	42.88
Carbohydrates	27.86	31.57
ME (MJ/kg)	12.88	13.13
Fatty acid groups (*%*)		
SFA	32.698	22.194
UFA	67.302	77.806
MUFA	38.123	31.674
PUFA	29.179	46.132
n-6 PUFA	26.369	33.525
n-3 PUFA	2.810	12.055
n-6/n-3 PUFA	9.389	2.781
PUFA/SFA	0.892	2.079

ME, metabolizable energy for pigs; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [1]1 kg of vitamin-mineral premix provided: vitamin A, 667,000 IU; vitamin D_3,110,000 IU; vitamin E, 2,800 IU; vitamin K_3, 130 mg; vitamin B_1, 140 mg; vitamin B_2, 470 mg; vitamin B_6, 195 mg; vitamin B_{12}, 280 µg; niacinamide, 1,445 mg; capantothenate, 1,000 mg; biotin, 5700 µg; choline Cl, 111,170 mg; $CuSO_4.5H_2O$, 1,100 mg; $FeSO_4.H_2O$, KI, 84 mg; MnO, 3,340 mg; ZnO, 10,000 mg; $Na_2O_3Se_1$, 34 mg; lysine, 331 g; methionine, 66 g; threonine, 142 g; tryptophan, 8 g; endo-1,4-beta-xylanase (EC3.2.1.8), 122100 VU; endo-1,3 (4)-beta-glucanase (EC3.2.1.6), 166500 VU.

Provisions preceding slaughter and measuring after slaughter: After fattening was finished, the barrows were transferred to a slaughterhouse at maximum distance of 40 km from the experimental stable. Animals were not fed on the day when they were slaughtered, water for drinking was available. After resting for at least 2 h, the barrows were slaughtered after previous stunning. Slaughterhouse provided data on lean meat content and backfat thickness that were collected during classification of carcasses according to the ZP method (Zwei-Punkt-Verfahren) of the SEUROP system (EU decision 2005/1/ES). Collection of meat and backfat samples was performed within 24 h after slaughter from the area between the second and the third last rib and a portable fridge was used for their transport to the laboratory. Drip loss was determined from weight change of 150 g of meat hanging in a bag at 5°C in intervals of 24 and 48 h after slaughter.

Analyses: The dry matter of meat was weighed after drying in oven at 105°C. For determination of meat firmness, Warner-Bratzler shear machine (Instron 3360, Canton, MA, USA) was used. Samples wrapped in PE bags were then stored at –20°C until further analyses. The thiobarbituric acid method of Piette and Raymond (1999) was used for determination of meat and backfat oxidative stability and results were expressed as amount of thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde per kg of meat. Intramuscular fat content was measured by extraction in the Soxtec 1043 device (FOSS Tecator AB, Hoganas, Sweden) in accordance with CSN ISO 1444 (1997). For measurement of *p*H in meat, a portable *p*H meter (*p*H 340i) equipped with a glass electrode was used and the measurement was performed in fresh samples, 45 min and 24 h post-mortem. Samples of intramuscular fat (collected from *M. longissimus lumborum et thoracis*) were used for determination of fatty acid composition after chloroform methanol extraction of total lipids was performed according to Folch *et al.* (1957). Fatty acid methyl esters were prepared according to CSN ISO 5509 (1994) and analysed by gas chromatograph (6890N Agilent Technologies) equipped with DB-23 cyanopropyl-methyl polysiloxane column (60 m × 0.25 mm × 0.25 µm) using nitrogen as carrier gas with flow rate 0.8 ml/min, according to CSN ISO 5508 (1994). Temperature regime during the procedure: 120°C–6 min, heating (15°C/min) to 170°C and then heating (3°C/min) to 210°C, this temperature was maintained for 13.5 min followed by heating (40°C/min) up to 230°C which was held for 7 min.

Temperature of the flame ionisation detector was 260°C. Comparison with standards (37 Component FAME Mix, PUFA No. 1, PUFA No. 2, PUFA No. 3; Sigma-Aldrich) was used for determination of the fatty acid profile and percentages of total fatty acid were calculated.

Statistical analysis: The data were statistically analysed in the form of mean±standard error by One way ANOVA and the Student's test in QC expert software (TriloByte Statistical Software Ltd.). The differences between means were considered very highly statistically significant (***) when P<0.001, highly statistically significant (**) when P<0.01 and statistically significant (*) when P<0.05. Data from the one level of linseed scrap were analysed as a completely randomized design. The analysis was in accordance with the following model:

$$y_{ij} = \mu + \alpha_i + e_{ij}$$

where, y_{ij}, the parameter; μ, overall mean; α_i, level of linseed scrap; e_{ij}, error term.

RESULTS AND DISCUSSION

Intramuscular fat was lower by 1.1% (P<0.05) in the pigs fed with the L mixture, than in the pigs from the C group (Table 2). Backfat thickness, lean meat content, drip loss value and *p*H of meat were not significantly influenced by feeding L diet. This may suggest the effect of pig genotype used (indigenous, resistant), which was also pointed out by Matoušek *et al.* (2016). The study of Beèková and Václavková (2010) performed on commercial type pigs fed with linseed diet (13.4%) from the weight of 39.00 kg to the weight of 89.40 did not prove a significant effect of this diet on an increase of intramuscular fat content, which corresponds to our findings. Similar results were described by Èítek *et al.* (2015) who fed L diet (150 g/kg) to pigs from 28.7 kg to 110 kg and also by Vaclavkova *et al.* (2016) whose observation included Prestice Black-Pied pigs from 72 kg for the duration of 56 days with 70 g of linseed per kg of feed mixture. Huang *et al*. (2008) proved that the longer supplementation of diet with linseed, the higher is intramuscular fat content. Evaluation of meat oxidative stability showed that the experimental group of animals were characterized by higher concentration of malondialdehyde on the 3rd day (P<0.01) and the 6th day than the C group of pigs. Vaclavkova *et al.* (2016) published similar findings of higher malondialdehyde concentration in experimental pigs. Most studies, such as Haak *et al.* (2008), Guillevic *et al.* (2009) and Jasiñska and Kurek (2017) say that enrichment of feeding mixture with n-3 PUFA leads in general to increased sensitivity of meat to oxidation, regardless of linseed form (oil, scrap, whole seed) or concentration. The studies of Sobotka *et al.* (2012) and Brodowska *et al.* (2018) highlight the relation between pork

Table 2. Characteristics of carcass, meat quality and oxidative stability in the control and linseed group (mean±standard error)

Specification	Control (C)	Linseed scrap (L)	Significance
Backfat thickness (mm)	21.69±0.31	21.37±0.43	ns
Lean meat content (%)	52.76±0.78	53.92±0.12	ns
Intramuscular fat (%)	2.97±0.13	1.87±0.12	*
Drip loss (%)	2.55±0.16	2.43±0.09	ns
pH_{45}	5.43±0.08	5.87±0.02	ns
pH_{24}	5.12±0.04	5.68±0.03	ns
TBARS (malondialdehyde) concentration (mg/kg)			
Day 1	0.04±0.00	0.06±0.01	ns
Day 3	0.06±0.00	0.10±0.02	**
Day 6	0.11±0.01	0.16±0.03	*

TBARS, thiobarbituric acid-reactive substances; ns, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001.

Table 3. Fatty acid profile (% of total fatty acids) in *M. longissimus lumborum et thoracis* of the control and linseed group (mean±standard error)

Fatty acid	Control (C)	Linseed scrap (L)	Significance
C6:0	0.006±0.001	0.011±0.001	**
C8:0	0.008±0.002	0.020±0.003	***
C10:0	0.059±0.003	0.051±0.002	ns
C12:0	0.063±0.019	0.068±0.002	ns
C13:0	0.005±0.001	0.006±0.001	ns
C14:0	1.127±0.054	1.077±0.026	ns
C15:0	0.106±0.052	0.053±0.002	ns
C16:0	22.984±0.446	21.577±0.457	*
C17:0	0.254±0.022	0.199±0.008	*
C18:0	11.955±0.350	10.738±0.319	*
C20:0	0.216±0.010	0.209±0.022	ns
C22:0	0.013±0.002	0.018±0.002	ns
C24:0	0.017±0.001	0.019±0.001	ns
C14:1 n-5	0.018±0.001	0.020±0.001	ns
C16:1 n-7	3.002±0.144	2.904±0.132	ns
C18:1 n-7	3.941±0.151	3.639±0.159	ns
C18:1 n-9	42.708±0.185	40.042±1.181	*
C20:1 n-9	0.697±0.038	0.760±0.062	ns
C24:1 n-9	0.033±0.002	0.041±0.004	ns
C18:2 n-6	9.564±0.718	11.370±0.022	ns
C20:2 n-6	0.304±0.018	0.338±0.010	ns
C18:3 n-3	0.468±0.023	2.313±0.372	***
C18:3 n-6	0.024±0.003	0.026±0.003	ns
C20:3 n-3	0.085±0.005	0.102±0.010	*
C20:3 n-6	0.201±0.010	0.222±0.016	ns
C20:4 n-3	0.019±0.001	0.033±0.004	**
C20:4 n-6	1.468±0.056	1.707±0.152	ns
C22:4 n-6	0.256±0.008	0.260±0.024	ns
C20:5 n-3	0.142±0.013	0.185±0.009	*
C22:5 n-3	0.284±0.016	0.375±0.032	*
C22:6 n-3	0.032±0.002	0.037±0.002	ns
SFA	36.814±0.738	34.046±0.761	*
UFA	63.186±0.738	65.954±0.761	*
MUFA	50.400±0.419	47.407±1.378	*
PUFA	12.787±0.821	18.547±1.914	*
n-6 PUFA	11.817±0.697	13.924±0.727	ns
n-3 PUFA	1.030±0.045	3.045±0.381	***
n-6/n-3 PUFA	11.612±0.717	5.178±0.645	***
PUFA/SFA	0.353±0.033	0.558±0.073	*

SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ns, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001.

meat oxidative stability and α-linolenic acid concentration. The higher content of this acid in meat, the more likely is a deterioration of oxidative stability, which was confirmed also by Jasiñska and Kurek (2017).

Regarding SFA, it was observed that feeding L diet led to an increase of C6:0 (P<0.01), C8:0 (P<0.001) and a decrease of C16:0, C17:0, C18:0 (P<0.05) in comparison to the C group (Table 3). From the group of MUFA, a significant decrease of C18:1 n-9 (P<0.05) was recorded in the experimental population. The most significant differences were found in PUFA, namely an increase of C18:3 n-3 (P<0.001), C20:3 n-3 (P<0.05), C20:4 n-3 (P<0.01) and also C20:5 n-3 a C22:5 n-3 (P<0.05) in the L group of pigs. This finding corresponded to higher content of n-3 PUFA (P<0.001) and more favourable n-6/n-3 PUFA ratio in the L group against the control. Higher PUFA/SFA ratio (P<0.05) was observed in the experimental group of pigs. The analysis of fatty acid profile in meat proved that linseed diet positively influences content of fatty acids. This was confirmed by Èervek *et al.* (2011), who fed 1.5% linseed diet to hybrid pigs from 25 kg to slaughter (110 kg) and recorded 1.6% increase of n-3 PUFA in meat of the experimental pigs and proved better n-6/n-3 PUFA ratio (12.6% control vs 5.5% experimental). Karolyi *et al.* (2012) used feed mixture with 3% of linseed scrap for 30 days and recorded 3% increase of n-3 PUFA. These results show that even lower concentrations of linseed in feed mixture positively influence fatty acid profile. In most studies, linseed concentration in feed mixture ranges from 5 to 15% and the increase of n-3 PUFA in meat is up to 3% in most cases (Wood *et al.* 2003, Huang *et al.* 2008, Petroviæ *et al.* 2014). However, Okrouhlá *et al.* (2013) observed 6.5% increase of n-3 PUFA in an experimental group of pigs with utilization of feeding mixture enriched in linseed in dose of 150 g/kg.

In conclusion, it can be said that feeding linseed diet to pigs can improve fatty acid profile in meat in favour of n-3 PUFA and at the same time improve the n3/n6 PUFA ratio. In this context, the pork meat has better usability potential.

ACKNOWLEDGEMENTS

This study was supported by the project of MENDELU internal grant agency, Faculty of Agriculture No. TP 7/2017 and Supported by the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO0718.

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