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Fatty acid and amino acid profiles in muscle *longissimus lumborum et thoracis* of the indigenous Prestice Black-Pied pig breed in comparison with a commercial pig hybrid

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ABSTRACT

The objective of the study was to analyse the fatty acid profile (FA) and amino acid profile (AA) in the *musculus longissimus lumborum et thoracis* (MLLT) in two contrast pig populations. The study was carried out on a total of 39 pigs (19 barrows and 20 gilts) of the indigenous Prestice Black-Pied Pig (PB) and 37 pigs (17 barrows and 20 gilts) of the three-breed commercial hybrid of Large White × Landrace × Large White_{sireline} (CH). The pigs were kept in the same conditions of diet and management. The slaughter was carried out at the average weight of 95 kg. Sex of the evaluated pigs was also taken into account. The CH pigs showed lower contents of saturated FA ($p < 0.01$), monounsaturated FA (MUFA; $p < 0.05$) and a higher content of *n*-6 polyunsaturated FA (PUFA; $p < 0.05$), more favourable health indexes, expressed as lower values of atherogenic ($p < 0.001$), thrombogenic ($p < 0.001$) and saturation (saturated/unsaturated FA) index ($p < 0.001$) and positively higher values of hypocholesterolemic/hypercholesterolemic FA ratio ($p < 0.01$). The gilts showed lower content of MUFA ($p < 0.05$). Significant ($p < 0.05$) interaction effects between genotype and sex were recorded for the caprylic acid, capric acid, lauric acid, tridecylic acid, myristic acid, stearic acid, arachidic acid, α -linolenic and γ -linolenic acid ($p < 0.01$), with the highest content found in PB barrows. Differences in the AA profiles in the MLLT muscle were only minimal. The PB pigs showed higher proportion ($p < 0.05$) of threonine, isoleucine and aspartic acid. Higher proportions of threonine and valine were observed in barrows ($p < 0.05$). The results indicate that the evaluated populations of pigs are characterised by significantly different composition of FA. The health indexes suggest more favourable values in the meat of CH pigs.

HIGHLIGHTS

- The aim of the study was comparison of fatty acid (FA) and amino acid (AA) composition in meat of two contrast pig genotypes.
- Regarding the FA profile, the modern hybrid showed more favourable health indexes than the Prestice Black-Pied Pig (PB).
- These two populations of pigs, kept in the same conditions, had similar composition of AAs in meat.

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Introduction

Pork is the most consumed type of meat in Europe. In the Czech Republic, it covers 51.7% of overall meat consumption, the most of all meat types. In 2020, the consumption of pork reached the level of 43.4 kg/person/year. Global pork consumption in 2020 was 107.2 million tons (Havlíček et al. 2021). However, the health risks associated with consumption of pork still remain a question (Šrédl

et al. 2021). In general, pork is considered a meat type less favourable in terms of its effects on human health, mainly in context of a higher content of saturated fatty acids (SFA), which are associated with cardiovascular problems. It has been reported that composition of fatty acids (FA) and amino acids (AA) influences organoleptic and nutritional qualities of meat. For consumers, the requirements for the content of essential AA needs to be fulfilled,

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since the content of essential AA is usually used for assessment of protein biological value. At evaluation of FA, several points of view collide. The processing industry demands pork with a low content of polyunsaturated FA (PUFA), as they play role in lipolytic and oxidative processes. On the other hand, a higher content of n-3 PUFA and monounsaturated FA (MUFA) can have a positive effect on human health (Wood et al. 2008; Briggs et al. 2017). Most often, the production of pork uses final production hybrids based on 3–4 breeds. These hybrids are characterised by a high growth rate and a high lean meat content with low content of backfat and intramuscular fat. Modern breeds or hybrids have displaced the original pig breeds that are not competitive in the current production system. However, it must be said that the original breeds represent an important biological source for new breeds, biodiversity protection and sustainable breeding (Lebret et al. 2015; Aboagye et al. 2020; Gan et al. 2020). It is worth noting, that consumers' interests in the origin of food and questions of local production have been increasing in recent years. At the same time, meat of the indigenous breeds can be characterised as richer in taste and can enrich the offer of traders (Wang et al. 2021). These breeds include Prestice Black-Pied Pig (PB), which is currently maintained in a close population in the Czech Republic and is not used for commercial hybridisation. This breed is included in the National Programme for Farm Animal Resources, which belongs to European Regional Focal Point for Farm Animal Genetic Resources. The breed is characterised by an average fertility, good longevity, strong constitution and adaptability (Matoušek et al. 2016; Nevřkła et al. 2017; Nevřkła and Václavková 2020). In our previous study, we documented that this breed shows worse values of feed conversion, lower growth intensity, worse carcass value traits, but better meat quality parameters, such as pH and drip loss value in comparison with a commercial hybrid (Nevřkła et al. 2021). Nevertheless, there is only a small number of studies on evaluation of FA profile and no study has been performed so far on AA profile in the population of PB pigs in comparison with a commercial hybrid. Therefore, the objective of this study was to analyse these two contrast pig populations in terms of FA and AA content in muscle *longissimus lumborum et thoracis* (MLLT). Modern hybrids are selected for high homogeneity within the sexes, however, in indigenous breeds such selection has not been applied (Nevřkła et al. 2021). Therefore, the sex of pigs was taken into account in this study, in order to verify any differences.

Materials and methods

The animals were kept in operational conditions of a production farm in the Czech Republic in accordance

with regulations of the European Union (Council Directive 2008).

Animals and housing

The study was carried out on a total of 39 pigs (19 barrows and 20 gilts) of the indigenous PB and 37 pigs (17 barrows and 20 gilts) of the three-breed commercial hybrid of Large White × Landrace × Large White_{sireline} (CH). From weaning to slaughter, the pigs were kept in equal conditions, on deep bedding. Straw was added twice weekly and removed after removal of animals. The floor space was 2 m² per one pig. Till Day 79, the pigs were divided by genotype and kept in two pens. From Day 80 to the end of fattening, the pigs were divided by genotype and sex and kept in four pens. The experiment was terminated when the pigs reached the average slaughter weight of 95 kg. With regard to the lower growth intensity of the PB pigs against the CH pigs, the age at the time of slaughter was 194 d in the PB pigs and 157 d in the CH pigs.

Feeding

Both water and diet for pigs were available *ad libitum*. The diet in liquid form was fed in troughs equipped with sensors that controlled replenishment. The composition of the diet is shown in Table 1.

Slaughter of pigs and preparation of samples

After termination of fattening, the pigs were transferred from the experimental facility to a slaughterhouse located within 40 km. The animals were left to rest for about 2 h before slaughtering. Then, they were slaughtered by electrical stunning (350 V, 4 A) and exsanguination. The carcass was processed and cooled to 4 °C. After 24 h, samples (150 g) of *musculus longissimus lumborum et thoracis* (MLLT) were collected between the second and the third last rib and transported in a portable fridge to the laboratory for further analysis.

The dry matter content in the samples was determined by drying in a hot air dryer at 105 °C for 4 h to a constant weight, when the samples were homogenised using a Grindomix GM 200 knife mill (Retsch, Haan, Germany). Intramuscular fat (IMF) content in dry matter was then analysed after extraction with petroleum ether on the Soxhlet 1043 device (FOSS Tecator AB, Höganäs, Sweden) as described in ISO 1444 (1996). Nitrogenous substances were determined using the

Table 1. Ingredients and nutrient composition of the diets .

	A1	A2	A3
Ingredients as fed basis (%)			
Wheat	36	42	46
Barley	25	30	32
Soybean meal, extracted	22	20	12
Corn	13	5	7
Salt	0.4	0.4	0.4
Monocalcium phosphate	0.5	0.5	0.5
Magnesium oxide	0.1	0.1	0.1
Amino acids and vitamins ^a	3	2	2
Nutrients (%)			
Dry matter	88.2	88.9	88.4
Fat	5.4	2.4	1.7
N-substances	17	16.1	14.3
Ash	4.9	4.5	4.3
ME (MJ/kg)	13.0	12.9	12.8

A1: diet fed to pigs with average live weight to 35 kg. A2: diet fed to pigs with average live weight to 60 kg. A3: diet fed to pigs with average live weight to 100 kg.

^a1 kg of vitamin-mineral premix provided: vitamin A, 667.000 IU; vitamin D3, 110.000 IU; vitamin E, 2.800 IU; vitamin K3, 130 mg; vitamin B1, 140 mg; vitamin B2, 470 mg; vitamin B6, 195 mg; vitamin B12, 280 µg; niacinamide, 1.445 mg; Ca pantothenate, 1.000 mg; biotin, 5700 µg; choline Cl, 111.170 mg; CuSO₄·5H₂O, 1.100 mg; FeSO₄·H₂O, KI, 84 mg; MnO, 3.340 mg; ZnO, 10.000 mg; Na2O3Se1, 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.

Kjeltec Auto 1030 Analyser (FOSS Tecator AB, Höganäs, Sweden), the values were subsequently converted to protein content in meat with a coefficient of 6.25.

Analysis of fatty acids and amino acids

Samples of IMF collected from the *MLLT* were used for analysis of FA composition after extraction with chloroform and methanol from total lipids according to Folch et al. (1957). Nonadecanoic acid (C 19:0) was used as an internal marker for quantification of FAs in samples. Alkaline trans-methylation of FAs was performed as described by Raes et al. (2003) and in accordance with CSN ISO 12966-2 (2017). Gas chromatography of FA methyl esters (FAMES) was carried out using an Agilent 6890 M chromatograph (Agilent Technologies, Inc., local distributor HPST Ltd., Prague, Czech Republic). The gas chromatograph was equipped with a cyanopropyl-methylpolysiloxan column DB23 (60 m × 0.25 mm × 0.25 µm) using nitrogen as a carrier gas with a flow rate of 0.8 mL/min, according to CSN ISO 12966-1 (2014). Temperature regime during procedure: 120 °C – 6 min, heating (15 °C/min) up to 170 °C followed by heating (3 °C/min) to 210 °C, this temperature was maintained for 13.5 min and then raised (40 °C/min) to 230 °C. This temperature was maintained for 7 min. Temperature of flame ionisation detector was 260 °C. The FAs were identified on the basis of retention time corresponding to the standards used (PUFA 1, PUFA 2 and PUFA 3, CRM-Supelco 37 Component FAME Mix; Supelco, Bellefonte,

PA, USA). The FAs were expressed in mg per 100 g of meat.

Total SFA, MUFA, PUFA, *n*-6 and *n*-3 and *n*-6/*n*-3 and PUFA/SFA ratios were calculated from the FA composition data. FA indexes were also calculated.

Atherogenic index (AI) indicates the relationship between the sum of main SFA and the sum of main types of unsaturated FA. The first being considered pro-atherogenic and the latter anti-atherogenic. The AI was calculated according to Ulbricht and Southgate (1991) as follows:

$$AI = (C12:0 + 4 \times C14:0 + C16:0) / [\Sigma MUFA + \Sigma n6 + \Sigma n3].$$

Thrombogenic index (TI) that indicates the tendency to formation of clots in blood vessels, can be described as a relationship between anti-thrombogenic and pro-thrombogenic FA. The TI was calculated according to Ulbricht and Southgate (1991) using the formula:

$$TI = (C14:0 + C16:0 + C18:0) / [0,5 \times \Sigma MUFA + (0,5 \times \Sigma n6) + (3 \times \Sigma n3) + (\Sigma n3 / \Sigma n6)].$$

Saturation index (SI) was also calculated using the equation designed by Ulbricht and Southgate (1991):

$$SI = (C14:0 + C16:0 + C18:0) / (MUFA + PUFA).$$

Selected AAs were used for calculation of hypocholesterolemic/hypercholesterolemic ratio (h/H), as suggested by Santos-Silva et al. (2002):

$$h/H = \left[\left(\sum C18:1n9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3 \right) / \left(\sum C14:0 + C16:0 \right) \right].$$

For determination of AA content, representative samples of *MLLT* were selected. The samples were homogenised and subjected to chemical analyses for determination of selected AA using automated AA analyser AAA 400 (INGOS Ltd., Prague, Czech Republic) equipped with an ion-exchange column. AAs were released from protein molecules by acid hydrolysis with 6 M hydrochloride acid. Chromatographic software ChromuLan (INGOS Ltd, Prague, Czech Republic) was used for evaluation. The AAs were expressed in mg per 100 g of meat.

Statistical analysis

The data were analysed in SAS version 9.1 software (SAS Institute Inc., Cary, NC, USA). Evaluation of individual effects used linear model (PROC GLM). Statistical significance (**p* < 0.05; ***p* < 0.01; ****p* < 0.001) of

individual factor entering the model was assessed based on the analysis of variance using the TYPE III sum of squares table. The significance of differences ($p < 0.05$) among the individual means of the least squares (LSM) within a genotype and sex was tested using the Tukey's test.

Testing of significant differences was carried out according to the following mathematical-statistical two-way analysis model: $Y_{ij} = \mu + d_i + s_j + (ds)_{ij} + e_{ij}$ where: Y_{ij} = value of the trait, μ = overall mean, d_i = effect of genotype ($i = 1, 2$), s_j = effect of sex ($j = 1, 2$), $(ds)_{ij}$ = combined effect of genotype and sex and e_{ij} = random residual. The data in the Tables are presented as the LSM and the standard error of the mean (SEM). One pig represented an experimental unit.

Results

Fatty acid profile

Table 2 documents composition of FA in *MLLT* muscle in dependence on genotype and sex of the pigs and interaction of the two factors. Thirteen SFA, seven MUFA and nine PUFA were found in the samples of meat. Regardless of the genotype and sex of the pigs, the most abundant were oleic acid, palmitic acid, stearic acid, linoleic acid, palmitoleic acid and myristic acid. Of the SFA, meat of the PB pigs had higher content of myristic acid (difference 9.2 mg; $p < 0.001$), palmitic acid (difference 73.55 mg; $p < 0.01$), heptadecanoic acid ($p < 0.001$), stearic acid (difference 50.43 mg; $p < 0.01$), behenic acid ($p < 0.05$) and lignoceric acid ($p < 0.001$). The meat of the CH pigs showed higher contents of pentadecylic acid ($p < 0.001$). Evaluation of MUFA revealed lower content ($p < 0.001$) of myristoleic acid in the meat of PB pigs against the meat of CH pigs. On the contrary, the meat of CH pigs contained lower amount of palmitoleic acid (difference 10.77 mg; $p < 0.01$), margaric acid ($p < 0.01$), oleic acid (difference 122.41 mg; $p < 0.05$), eicosenoic acid ($p < 0.001$) and nervonic acid ($p < 0.01$) than the meat of PB pigs. Evaluation of PUFA showed higher concentrations of linoleic acid (difference 11.3 mg; $p < 0.05$) and arachidonic acid ($p < 0.05$) in the meat of hybrid pigs. Table 2 also shows groups, ratios and sums of FAs in the *MLLT* muscle. The meat of CH pigs contained less SFA (difference 141.68 mg; $p < 0.01$), less MUFA (difference 145.26 mg; $p < 0.05$) and more n-6 PUFA (difference 15.77 mg; $p < 0.05$). It also showed more favourable health indices, expressed as lower values of atherogenic ($p < 0.001$), thrombogenic ($p < 0.001$) and saturation (saturated/unsaturated FA) ($p < 0.001$) index and

positively higher values of hypocholesterolemic/hypercholesterolemic FA ($p < 0.01$).

The effect of sex was confirmed to be significant for some SFA. The gilts showed lower levels of capric acid ($p < 0.05$), stearic acid (difference 35.54 mg; $p < 0.05$) and arachic acid ($p < 0.01$). On the contrary, in barrows, lower levels of behenic acid ($p < 0.05$) and lignoceric acid ($p < 0.01$). As for MUFA, the effect of sex was significant for nervonic acid ($p < 0.01$) with lower values found in barrows. As for PUFA, the gilts showed lower levels of linoleic acid (difference 12.13 mg; $p < 0.05$) and γ -linolenic acid ($p < 0.001$). Evaluation of groups, sums and ratios of FAs revealed significant effect on content of MUFA ($p < 0.05$) with values 111.42 mg lower in gilts.

Significant interaction effect between genotype and sex was proved for caprylic acid ($p < 0.05$), with the lowest values found in meat of the CH barrows (1.15 mg) against the highest values in PB meat (2.55 mg). Also for capric acid ($p < 0.05$), with the lowest content found in the PB gilts (1.67 mg) and the highest in the PB barrows (3.96 mg). As same as for lauric acid (PB gilts 1.95 mg vs. PB barrows 4.21 mg; $p < 0.05$) and tridecylic acid (PB gilts 1.40 mg vs. PB barrows 3.01 mg; $p < 0.05$). Another interaction between factors was found for myristic acid ($p < 0.001$), with the highest content recorded in the PB barrows (23.52 mg) against the lowest in the CH barrows (10.02 mg). A significant interaction was also recorded for arachic acid ($p < 0.05$) with the lowest content found in the PB gilts (2.21 mg) and the highest in the PB barrows (4.48 mg). As for γ -linolenic acid ($p < 0.01$) the lowest content was observed in the PB gilts (0.44 mg) and the highest in the PB barrows (1.86 mg). Same trend was found for the α -linolenic acid ($p < 0.05$) with the highest levels found in the PB barrows (10.83 mg) and the lowest levels found in the PB gilts (7.75 mg).

Amino acid profile

Table 3 presents composition of AA in *MLLT* of pigs in dependence on genotype, sex and mutual interaction of the given factors. The genotype affected content of AAs only minimally. The significant differences were recorded for the contents of threonine, isoleucine and aspartic acid ($p < 0.05$), with highest contents found in the meat of PB pigs.

Sex affected the contents of threonine and valine ($p < 0.05$) with higher values found in barrows.

No significant interactions were found for the contents of essential and semi-essential AA.

Table 2. Fatty acid profile of *musculus longissimus lumborum et thoracis* muscle depending on genotype and sex (mg/100g).

Item	Genotype (G)				SEM	Significance		
	PB		CH			G	S	G × S
Sex (S)	Gilts	Barrows	Gilts	Barrows				
C6:0 (Caproic)	2.34	2.36	1.85	1.62	0.30	ns	ns	ns
C8:0 (Caprylic)	1.16 ^a	2.55 ^b	1.64 ^a	1.15 ^a	0.44	ns	ns	*
C10:0 (Capric)	1.67 ^a	3.96 ^b	3.25 ^b	3.08 ^b	0.47	ns	*	*
C12:0 (Lauric)	1.95 ^a	4.21 ^b	4.00 ^b	3.11 ^b	0.57	ns	ns	*
C13:0 (Tridecylic)	1.40 ^a	3.01 ^b	2.70 ^b	2.14 ^b	0.43	ns	ns	*
C14:0 (Myristic)	16.07 ^a	23.52 ^b	11.12 ^a	10.02 ^a	1.96	***	ns	*
C15:0 (Pentadecylic)	1.14	1.83	1.55	1.86	0.30	***	ns	ns
C16:0 (Palmitic)	231.56	299.87	185.63	199.41	24.26	**	ns	ns
C17:0 (Margaric)	5.44	6.03	3.26	3.07	0.56	***	ns	ns
C18:0 (Stearic)	118.27 ^a	180.87 ^b	97.12 ^a	101.39 ^a	13.34	**	*	*
C20:0 (Arachidic)	2.21 ^a	4.48 ^b	2.75 ^a	2.78 ^a	0.41	ns	**	*
C22:0 (Behenic)	6.83 ^b	4.36 ^{ab}	4.46 ^{ab}	2.98 ^a	0.76	*	*	ns
C24:0 (Lignoceric)	9.68	6.07	4.59	3.81	0.72	***	**	ns
C14:1n5 (Myristoleic)	0.40	0.46	1.29	1.58	0.19	***	ns	ns
C15:1n5 (Pentadecenoic)	4.01	5.57	3.65	3.65	0.67	ns	ns	ns
C16:1n7 (Palmitoleic)	35.17	48.08	30.20	31.60	3.71	**	ns	ns
C17:1n7 (Heptadecenoic)	5.12	5.76	3.38	3.20	0.56	**	ns	ns
C18:1n9 (Oleic)	388.27	557.79	337.77	364.89	47.43	*	ns	ns
C20:1n9 (Eicosenoic)	11.52	14.84	6.69	5.59	1.11	***	ns	ns
C24:1 (Nervonic)	8.30	4.55	4.19	3.07	0.75	**	**	ns
C18:2n6 (Linoleic)	68.55	88.78	87.97	92.18	5.62	*	*	ns
C20:2n6 (Eicosadienoic)	4.06	5.51	5.06	3.20	0.80	ns	ns	ns
C18:3n6 (γ-linolenic)	0.44 ^a	1.86 ^b	1.60 ^b	1.55 ^b	0.22	ns	**	**
C18:3n3 (α-linolenic)	7.75 ^a	10.83 ^a	10.03 ^a	9.23 ^a	0.85	ns	ns	*
C20:3n6 (Dihomo-γ-linolenic)	3.41	4.41	4.65	3.17	0.59	ns	ns	ns
C20:3n3 (Eicosatrienoic)	1.98	2.32	2.64	3.01	0.33	ns	ns	ns
C20:4n6 (Arachidonic)	10.51	10.62	16.20	14.05	1.20	***	ns	ns
C20:5n3 (Eicosapentaenoic)	2.06	1.81	2.64	1.40	0.54	ns	ns	ns
C22:6n3 (Docosahexaenoic)	2.30	2.50	3.25	1.67	0.55	ns	ns	ns
SFA	399.72	543.12	323.72	336.44	40.22	**	ns	ns
MUFA	452.80	637.06	381.17	413.57	52.27	*	*	ns
PUFA	101.07	128.64	134.03	129.45	8.58	ns	ns	ns
n-6	86.97	111.18	115.47	114.15	7.17	*	ns	ns
n-3	14.10	17.47	18.56	15.30	1.78	ns	ns	ns
n-6/n-3	6.73	6.60	7.28	7.70	0.44	ns	ns	ns
PUFA/SFA	0.27	0.27	0.46	0.41	0.02	*	ns	ns
AI	0.56	0.52	0.43	0.44	0.02	***	ns	ns
TI	1.23	1.17	0.93	0.99	0.05	***	ns	ns
SI	0.70	0.67	0.55	0.56	0.03	***	ns	ns
h/H	2.00	2.26	2.47	2.39	0.09	**	ns	ns
IMF (%)	2.20	2.38	1.58	1.64	0.04	***	ns	ns

PB: Prestice Black-Pied pig; CH: commercial hybrid; SEM: standard error of the mean; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; AI: atherogenic index; TI: thrombogenic index; SI: saturation index; h/H: hypocholesterolaemic/hypercholesterolaemic ratio; IMF: Intramuscular fat.

Results of the variance analysis are indicated as significant (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$) or not significant (ns).

^{a,b}Mean values in the same row marked with a different superscript indicate statistical significance ($p < 0.05$).

Discussion

Fatty acid profile

The performed experiment confirmed a significant effect of pig genotype on the twenty detected FA in *MLLT*. In the group of SFA, the PB pigs showed significantly higher contents of majority of FA, mainly myristic acid, palmitic acid and stearic acid. Zhang et al. (2019) also proved significantly higher content of myristic acid (1.38 mg/g), palmitic acid (27.29 mg/g) and stearic acid (12.61 mg/g) in meat of the indigenous Jiaying Black (JB) pig (IMF 4.8%) than in Duroc (D) × Landrace (L) × JB hybrids (IMF 4.72%; myristic acid 1.14 mg/g, stearic acid 26.32 mg/g and palmitic acid 11.79 mg/g) and in the commercial Berkshire breed (B)

(IMF 3.01%; myristic acid 1.12 mg/g, stearic acid 27.00 mg/g and palmitic acid 12.11 mg/g). The study led by Wood et al. (2008) suggests that there is a certain regularity in composition of FA in muscles despite the effects of many factors. The authors suppose that myristic and palmitic acid are synthesised FA, therefore higher contents in fatter pigs can be expected, which corresponds to the findings of our study. However, Wang et al. (2021), who evaluated a commercial hybrid PIC (slaughter weight 130 kg; IMF, 6.24%) and Large Black pig (slaughter weight 121 kg; IMF 10.02%) did not confirm a relationship between the hybrid or the indigenous breed and representation of individual SFA in meat, except for capric acid. The meat of PB pigs also contained higher levels of MUFA, mainly

Table 3. Amino acid profile of *musculus longissimus lumborum et thoracis* muscle depending on genotype and sex (g/100 g).

Item	Genotype (G)				SEM	Significance		
	PB		CH			G	S	G × S
	Gilts	Barrows	Gilts	Barrows				
Sex (S)								
Threonine	3.90	4.31	3.71	3.88	0.14	*	*	ns
Valine	3.94	4.33	3.89	4.06	0.12	ns	*	ns
Methionine	2.31	2.31	2.35	2.38	0.05	ns	ns	ns
Isoleucine	3.81	3.98	3.59	3.70	0.12	*	ns	ns
Leucine	5.57	6.90	6.40	6.68	0.20	ns	ns	ns
Phenylalanine	3.18	3.19	3.08	3.32	0.13	ns	ns	ns
Lysine	6.86	6.94	6.50	6.61	0.17	ns	ns	ns
Histidine	3.39	3.57	3.31	3.41	0.09	ns	ns	ns
Arginine	5.20	5.55	4.98	5.24	0.15	ns	ns	ns
Aspartic acid	7.94	8.44	7.41	7.79	0.24	*	ns	ns
Serine	3.33	3.60	3.14	3.27	0.11	ns	ns	ns
Glutamic acid	13.55	14.11	12.93	13.33	0.40	ns	ns	ns
Proline	4.04	4.39	3.84	4.16	0.24	ns	ns	ns
Glycine	3.70	3.81	3.45	3.56	0.11	ns	ns	ns
Alanine	4.62	4.78	4.31	4.58	0.14	ns	ns	ns
Tyrosine	2.76	3.05	2.72	2.84	0.11	ns	ns	ns
EAA	39.16	41.08	37.82	39.28	1.06	ns	ns	ns
nonEAA	39.91	42.18	37.80	39.54	1.20	ns	ns	ns
Protein (%)	20.20	20.04	23.15	21.96	0.33	***	ns	ns

PB: Prestice Black-Pied pig; CH: commercial hybrid; SEM: standard error of the mean; EAA: essential amino acids; nonEAA: non-essential amino acids.

Results of the variance analysis are indicated as significant (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$) or not significant (ns).

palmitoleic, oleic and eicosaenoic acid. Most studies are in accordance with our results (Zhang et al. 2019; Wang et al. 2021). For example, Tomović et al. (2016) described significantly lower content of these FA in *MLLT* of Large White (LW) (slaughter age 244 d; 154 kg; IMF 2.56%) than in Mangalitsa (M) breed (slaughter age 523 d; 150 kg; 5.86%) and hybrids of M × D (slaughter age 364 d; 154 kg; 4.32%). Similarly, Aboagye et al. (2020) recorded significantly higher content of palmitoleic, heptadecenoic and eicosaenoic acid in back muscle of a local breed Apulo-calabrese (135 kg; IMF 2.09%) against the commercial hybrid LW × L × D (155 kg; IMF 1.68%). For oleic acid, the difference was not statistically significant. As for the group of PUFA, a significantly higher content of linoleic acid and arachidonic acid was found in the CH pigs. Linoleic acid is the main compound of PUFA, takes part in many metabolic reactions and can have an anticarcinogenic effect. Linoleic acid is a source for synthesis of arachidonic acid, which is a precursor for synthesis of eicosanoids (Williams 2000). The results shown in this study are in line with the findings of Wang et al. (2021), who found higher contents of these FA in the commercial hybrid PIC than in the Large Black pigs. Debreceni et al. (2018) also recorded significant differences between populations of pigs (slaughtered at 100 kg) in content of linoleic acid in dependence on IMF percentage, while in the Slovakian population of Large White pig (IMF 1.2%)

they found 8.87% of this FA and in the M breed (IMF 1.9%) it was 9.16%, in the Polish breeds Pulawska (IMF 5.5%) (linoleic acid percentage 6.16%) and in the breed Zlotnicka spotted (IMF 2.6%) it was 10.85%. From the conclusions of Pietruszka et al. (2015), it can be stated, that differences in FA profile are frequently associated with different IMF content, which has been described above. However, there are studies where the IMF content did not differ between the populations and even despite being fed with the same compound feed, the animals showed significant differences in composition of FA. Which suggests that different pig populations have different expression of genes coding desaturation enzymes and thus influencing the FA profile (Robina et al. 2013; Yu et al. 2013; Davoli et al. 2019). A close positive relationship between muscle malic enzyme activity and IMF deposition was reported, both of which were higher in local breeds. Higher lipogenic capacity was also indicated by the activities of acetyl-CoA carboxylase, malic enzyme, glucose-6-phosphate dehydrogenase in *semimembranosus* muscle, which was found to be significantly higher in indigenous Basque compared to in the modern Large White breed (Alfonso et al. 2005). Similarly, Mourot and Kouba (1998) state that activity of the same enzymes in *semimembranosus* muscle was higher in the indigenous Meishana breed in comparison with the modern Large White breed. The study conducted by Zhao et al. (2017) confirmed a lower

activity of hormone-sensitive lipase hormone in Wujin pigs. According to Poklucar et al. (2020), it can be assumed that lipogenesis is elevated and lipolysis is restrained in fatty indigenous breeds compared to lean commercial pig breeds. The results focussed in sums and ratios of FA showed significant differences between the evaluated groups. There were significantly less SFA and MUFA and significantly more n-6 PUFA in the meat of CH pigs compared to the meat of PB pigs. The PUFA/SFA ratio was significantly lower in the PB pigs. As same as in this study, Teixeira and Rodrigues (2013) described significantly higher content of SFA and MUFA and lower PUFA in Preto Alentejano pig (90 kg; IMF 4.94%) and lower content of PUFA than in the L × LW hybrid (90 kg; IMF 3.40%). Similar results were presented also by Zhang et al. (2019) in the meat of indigenous JB pigs, D × L × JB hybrids (more SFA and MUFA and more PUFA) and the commercial breed (B) (less SFA and MUFA and more PUFA). Generally, in most studies, a higher content of MUFA and a lower content of PUFA was confirmed in the autochthonous breeds. Nevertheless, for SFA, the conclusions are ambiguous (Tomović et al. 2016; Debrecéni et al. 2018). The differences between PB pigs and CH pigs can be caused by different growth and adipogenous potential of the observed populations of pigs. Overall, these results are consistent with the fact that the lower fat deposit in body, the higher PUFA percentage and the lower SFA and MUFA share and vice versa (Wood et al. 2008; Jacyno et al. 2015). Cameron et al. (2000) showed that contents of C18: 2, C20: 4 and C22: 6 PUFAs were in a positive correlation with flavour of meat. Unsaturated FAs provide the primary substrate for oxidative processes, and an increase of n-3 PUFA content, in particular, may reduce the oxidative stability of pork and consequently diminish its sensory properties (Kristinsson et al. 2001; Karolyi et al. 2012). This suggests more favourable potential of the PB meat. For evaluation of the meat from the health perspective, more important are the indexes of FAs than concentrations of individual FAs. For example, AI and TI reflect probability of pathogenic phenomena, such as formation of atheroma and thrombi (Englmaierová et al. 2020). Results of the given indexes show that meat of the hybrid pigs is more favourable than meat of the PB pigs. Similar results were recorded also for a Lithuanian Indigenous Wattle breed in comparison with a commercial LW breed (Razmaitė et al. 2021).

The experiment confirmed effect of sex on contents of some SFA. The barrows had significantly more capric and stearic acid in their *MLLT*, while the gilts had

higher levels of behenic acid and lignoceric acid. From MUFA, only the content of nervonic acid was affected, with higher values found in gilts. From PUFA, a higher content of linoleic and γ -linolenic acid was observed in barrows. Evaluation of overall sum and ratios confirmed the effect of sex only on the content of MUFA, with higher values recorded in barrows. Some authors also described effect of sex on some FA. For example, Garitano et al. (2013) stated that meat of L × LW × D barrows (slaughter weight 126 kg; IMF 6.48%) has significantly higher content of myristic acid and palmitic acid when compared to the gilts (slaughter weight 124 kg; IMF 4.88%), which was not confirmed in our observation. However, same as in our experiment, the authors found significantly higher content of MUFA in barrows against the gilts. Kasprzyk et al. (2015) evaluated the populations of Pulawska pig and Polish L and found minimal differences in the contents of individual SFA in *MLLT* between the sexes. They found only a higher content arachic acid in barrows, which was confirmed in this study, in overall evaluation of SFA, the barrows showed a significantly higher value. This trend was evident also in our experiment. In both the observed populations, the gilts had higher contents of palmitoleic acid from MUFA, no significant differences between the sexes were found for PUFA. On the contrary, Lorenzo et al. (2012) evaluated the Celta pigs slaughtered at 114.2 kg and observed higher contents of majority of SFA in the IMF, therefore a higher overall SFA content and a lower PUFA content in gilts, in comparison with barrows. The given findings imply that there are great differences between sexes of the described pig populations that are kept in different states. It is obvious that local conditions, direction of pig breeding and breeding systems together with nutrition play a great role here. The content of FAs in adipose tissue of pigs is, according to Doran et al. (2006), affected by lipogenic enzymes, manifested in dependence on sex. Thickness of the subcutaneous fat differs between gilts and boars, due to differences in metabolism of sex hormones (Nürnberg et al. 1998). The effect of castration on the content of FAs in adipose tissue of barrows was described by Nuernberg et al. (2005). In their study, the gilts showed higher content of PUFA in IMF than the barrows, regardless to the source of fat in feed ration. Also, the study of Alonso et al. (2009) confirmed a higher content of PUFA in IMF of gilts than of barrows. In the observed population of pigs, significant interactions were found between the factors, namely in the group of individual SFA and some PUFA. Similarly, Teixeira and Rodrigues (2013) recorded significant interactions between the

pig populations (Preto Alentejano and a commercial hybrid) and sex (barrows and gilts), namely for lauric acid, myristic acid, palmitic acid, stearic acid and linoleic acid. Kim et al. (2020) also proved significant interactions between breed (D and P) and sex (barrows and gilts) for some SFA, namely capric acid and lauric acid and for some PUFA, specifically eicosadienoic and α -linolenic acid. It is noticeable, that the PB barrows showed the highest content of C18:3n3, which was unexpected. Some studies suggest that the differences might be related to the genetic background of the pigs, Davoli et al. (2019) state that the differences in the FA profiles of meat may be attributed to genetic and epigenetic effects or to causal interactions between genes and their products. For example, Dal Bosco et al. (2012), who analysed chicken meat, found that the slow-growing strains seem to have a higher efficiency of eicosapentaenoic acid and docosahexaenoic acid deposition in comparison with meat-type chickens. Robina et al. (2013) recorded a significant interaction (breed \times sex) for SFA, in animals slaughtered at the age of 194 d, however, no such significant interaction was found in animals slaughtered at the age of 227 d. For MUFA, the results were contrarywise. However, there are studies that found only minimal or no significant interaction effects of genotype and sex of pigs (Kasprzyk et al. 2015).

Amino acid profile

The population of pigs affected the AA profile only minimally. Statistically significant differences were observed only for threonine and isoleucine. In the group of non-essential AA, differences were found only for aspartic acid. AAs are the basic units of proteins and composition of proteins plays a vital role in meat quality by carrying the nutritional value and taste properties. Jiang et al. (2011) state that the *MLLT* muscle of Chinese breeds Dahe and Dawu is characterised by high contents of lysine, leucine, glutamine and aspartic acid, which corresponds to our findings. However, the authors found no significant differences in proportions of AA between the breeds. Contrarywise, Zhang et al. (2019), who evaluated a wide population of pure breeds (JB, slaughter weight 71.77 kg; B, slaughter weight 138.06 kg) and hybrids (B \times JB, slaughter weight 86.39 kg; D \times B \times JB, slaughter weight 108.39 kg; D \times L \times JB, slaughter weight 106.28 kg) found significant differences for all the AA. The recorded differences could be related to different slaughter weight of pigs. This relation is emphasised by Gan et al. (2020), who confirmed a relationship between composition of

individual AA and live weight in Liangshan pigs, with increasing weight, content of the essential AAs increased. Nevertheless, Zhang et al. (2019) proved differences even between the groups of hybrid pigs with very similar slaughter weight. It is evident, that more observation needs to be performed, since the conclusions are often ambiguous and available data are still lacking. Jiang et al. (2011) emphasise, that aspartic acid and glutamine are important taste precursors. It is noticeable that the PB pigs showed a significantly higher content of aspartic acid and a trend of higher glutamine was recorded.

The effect of sex on composition of AA was very limited, more threonine was found in barrows than in gilts, other AAs were not affected. Okrouhlá et al. (2006) also recorded minimal differences between gilts and barrows. Similarly, Thomas et al. (2019) found no significant differences in composition of AA between gilts and barrows of ghungroo \times H \times D hybrids. However, Cai et al. (2010) evaluated boars and barrows and conclude, that castration can have an effect on deposit of AA in *MLLT* muscle, i.e. that composition of AA can differ between castrated and uncastrated pigs. The barrows had lower levels of essential AA including isoleucine, lysine, methionine, phenylalanine, tyrosine, tryptophan, leucine, arginine and valine. Non-essential AAs were also higher in meat of barrows, including alanine, glycine, serine and asparagine in comparison with boars.

Conclusions

The performed study proved that even if the PB pigs are stabled and fed in the same manner as a commercial hybrid, the FA profile in *MLLT* differs significantly. Meat of the PB pigs was characterised with higher contents of SFA and MUFA, which documents that the meat could have more stable character in terms of fat oxidation. On the contrary, the *MLLT* of the CH pigs showed more favourable health indexes. It has been confirmed that sex of pigs affects only individual FAs, the effect of interaction between genotype and sex was also proven only for individual FAs, mainly from the SFA group. However, an unexpected finding was significantly the highest content of α -linolenic FA in the meat of PB barrows. Evaluation of the AA profile revealed similar composition of AAs in *MLLT* of PB pigs and CH pigs. The AA profiles were affected by sex of pigs only minimally. More studies are necessary to improve knowledge on biological processes of FA and AA synthesis in indigenous and commercially used pig breeds.

Ethical approval

The experiment was approved by the Ethics Committee of Mendel University Brno, Czech Republic (accreditation no. 57199/2020-MZE-18134).

Disclosure statement

No potential conflict of interest was reported by the author(s). The authors alone are responsible for the content and writing of this article.

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Data availability statement

The data presented in this study are available on request from the corresponding author upon reasonable request.

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