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Characterization of meat traits and fatty acids profile from Swallow-Belly Mangalitsa, Moravka pigs and their crossbreeds

ŁUKASZ MIGDAŁ[1], ČEDOMIR RADOVIĆ[2], VLADIMIR ŽIVKOVIĆ[2], MARIJA GOGIĆ[2], ANNA MIGDAŁ[3], WŁADYSŁAW MIGDAŁ[4]
[1] Faculty of Animal Science, University of Agriculture in Kraków
[2] Institute for Animal Husbandry in Belgrade
[3] Faculty of Animal Science, University of Agriculture in Kraków
[4] Faculty of Food Technology, University of Agriculture in Kraków

Abstract: *Characterization of meat traits and fatty acids profile from Swallow-Belly Mangalitsa, Moravka pigs and their crossbreeds.* Autochthonous breeds are among the most valuable sources of high quality meat for local products. In a recent study, we have analysed meat and fatty acids profile from Moravka, and Swallow-Belly Mangalitsa breeds, and from their crossbreed. Moreover, molecular analyses of *MC4R* and *LEP* gene were conducted for those breeds. We found previously identified mutation within the *MC4R* gene – c.892A > G (Asp298Asn). Statistical analysis did not show differences between genotypes for either Moravka or Swallow-Belly Mangalitsa meat chemical traits, shear force parameters or fatty acids profile. We found statistical differences in intramuscular fat content (%) between Moravka and crossbreed animals (10.77 ±4.28, and 6.76 ±1.31, respectively). For fatty acids profile, Swallow-Belly Mangalitsa was characterized by statistically the highest level of n-6 and n-3 PUFA (7.771 ±0.728, and 0.416 ±0.038, respectively). CLA level were the highest in Moravka, and the lowest in Swallow-Belly Mangalitsa (0.079 ±0.010, and 0.072 ±0,007, respectively). Our data showed that Moravka and Swallow-Belly Mangalitsa breeds can be valuable sources of meat, characterized by good quality.

Key words: Meat traits, Pig, Swallow-belly Mangalitsa, Moravka, fatty acids profile, LEP, MC4R

INTRODUCTION

Recently, the interest in native breeds has increased, both in the context of gene preservation and also the production of meat products manufactured in a traditional way. One of most well known native pigs in Europe is Mangalitsa, which is a typical fat breed farmed mainly in Hungary, and also in Serbia and Croatia. Carcass of this pig is characterized by 65–70% of fat in carcass sides, and approx. 30–35% of meat (Egerszegi et al. 2003). Results of recent studies (Egerszegi et al. 2003) show that this amount of meat is sufficient for the production of high quality ham. One of the reasons for high quality of ham is the traditional technology of rearing, as well as traditional nutrition (Petrović et al. 2010). Moravka is a breed of combined production, with more meat in carcass sides, and a breed of significantly less fat in Serbia (Petrović et al. 2010). This breed is reared in extensive conditions – both in terms of nutrition and housing. Moreover, the rearing and the lack of systematic selection of animals affect

the production traits, which are varied. On the other hand, this also results in these pigs' excellent adaptability, good vitality, and high resistance to diseases (Petrović et al. 2007).

Recently, molecular tools are used in the identification of polymorphisms within candidate genes for fatness traits, and their possible implementation into animal husbandry. One of the key factors controlling the energy balance is the Melanocortin 4 receptor (*MC4R*). As a part of leptin – signaling pathway, it decreases feed intake (Seeley et al. 2004). Known sequence variants of the *MC4R* gene reported in humans lead to obesity. Leptin gene, known also as the Ob or Obese is 146 amino acids expressed in adipose tissue (D'Andrea et al. 2007). Leptin, besides being a regulator of excessive fat deposition, seems to play an important role during the adaptation of animals to, as a GH secretion suppressor (Baratta et al. 2002), and attainment of puberty (Cunningham et al. 1999). In recent years, studies have been conducted in order to determine the association between reported polymorphism in the *LEP*, and carcass traits in animals (Jiang and Gibson 1999, Kennes et al. 2001, Oliveira de P.J. et al. 2006).

The goals of the present study included the chemical analysis of the samples, fatty acids profile, texture, and shear force analysis from m. *longissimus dorsi lumborum*. Moreover, we sequenced the *MC4R* and the *LEP* gene, in order to analyse the potential polymorphisms and their association with the studied traits.

MATERIALS AND METHODS

Animals

In the present study, we analysed m. *longissimus dorsi lumborum* between 13th and 14th rib samples from 20 Moravka (MO) pigs (12 males and 8 females), 18 Swallow-Belly Mangalitsa (SBM) pigs (10 males and 8 females) and crossbreed animals of Moravka × Swallow-Belly Mangalitsa (3 males and 5 females). All animals were slaughtered at the age of 365 days. Animals of both breeds were born and reared on Experimental pig farm of the Institute for Animal Husbandry, Belgrade-Zemun. The surface of the free range was 150 m^2 (110 m^2 open section and 40 m^2 covered section of the range). Fed composition is presented in Table 1

Meat analysis

Instrumental measurement of shear force

Cylinder-shaped samples (14 mm in diameter and 15 mm in height) were cut from the meat, and roasted in the oven at 180ºC until internal temperature of 78ºC. Shear force was measured using a TA-XT2 Texture Analyser (Stable Micro Systems), with a Warner-Bratzler attachment and a triangular notch in the blade. The blade speed during the test was 1.5 mm/s. Results were presented as force per area (kG/cm^2).

Instrumental measurement of texture parameters

Cylinder-shaped samples (14 mm in diameter and 15 mm in height) were

TABLE 1. Composition of pigs diet

Feedstuffs/the nutrient (%)	Fattening pigs	
	25–60 (kg)	60–100 (kg)
Corn (silage)	62.93	68.76
Wheat bran	15.00	15.00
Soybean oil meal	14.00	9.10
Sunflower meal	5.00	4.00
Limestone (calcium carbonate)	1.40	1.40
Monocalcium phospate	0.60	0.70
Salt	0.40	0.45
Premix	0.50	0.50
L-lysine	0.07	0.09
Zeolite	0.10	–
Total	100.00	100.00
Crude protein	15	13
ME, MJ/kg	12.95	13.05
Ether extracts	3.48	3.62
Fiber	4.21	3.87
Ash	5.01	4.88
Ca	0.70	0.70
P	0.60	0.59
Na, Sodium	0.23	0.24
Lysine	0.763	0.648
Met + cystin	0.557	0.506
Tryptophane	0.187	0.158
Threonine	0.581	0.503
Isoleucine	0.665	0.566
Valine	0.796	0.697
Arginine	1.012	0.848
Histidine	0.427	0.377
Leucine	1.436	1.314
Tyrosine + phenilalanine	1.404	1.240

cut from the meat roasted as described above. The texture was analysed using a TA-XT2 Texture Analyser (Stable Micro Systems), with an attachment in the form of a cylinder, 50 mm in diameter. The samples were subjected to a double pressing test using a force of 10 g to 70% of their height. The cylinder speed was 2 mm/s, and the interval between the pressures was 3 s.

Collagen content

The total collagen content was estimated according to Polish Standard PN-ISO 3496. The absorbance of samples was measured using the Novasina spectrophotometer, at 558 nm. The hydroxyproline content was read from the calibration curve. The total collagen content was calculated from hydroxyproline amount, using the coefficient of 7.25, and taking into account the dilution factors. The soluble collagen was calculated as the difference between the total collagen, and the insoluble collagen. The amount of insoluble collagen was estimated according to the method proposed by Liu et al. (1994), with our modifications.

The hydroxyproline content was read from the spectrophotometric curve. Insoluble collagen content was calculated using the coefficient of 7.25. The amount of soluble collagen was calculated and expressed as percentage of total collagen.

Meat quality traits

The meat samples were chemically analyzed to determine water, protein, intramuscular fat, and ash content according to Polish standards. Water content was

determined by the drying method in 105°C were calculated (PN-ISO-1442), protein content (%) by the method of Kjeldahl where protein content (%) were calculated as 6.25 × total nitrogen level from sample (PN-A-04018:1975), intramuscular fat content (%) by the method of Soxhlet petroleum- ether extraction using SOXTEK HTZ-2 (Tecator) (PN-ISO-1444), and total ash content (550°C for 12 hours) in accordance with PN-ISO-936.

Fatty acid profile

Samples were extracted with chloroform-methanol (2 : 1, v/v), according to the method developed by Folch et al. (1957). Then, 1 g of meat samples was mixed with 15 mL of chloroform-methanol mixture, and homogenized for 10 min at 5,000 rpm, and after a 5-minute pause – for another 5 min, at 1,000 rpm, using homogeniser MPW-120. The mixture was then filtered through filter paper to a regular cylinder, and completed with extraction mixture up to 15 mL. Next, 3 mL of 0.74% KCl solution was added to 15 mL of the filtrate. The alcohol–water phase was removed, and the chloroform phase was washed three times using 2 mL solution of chloroform : methanol : 0.74% KCl (3 : 48 : 47, v/v/v). Subsequently, the chloroform phase was recovered, dehydrated with anhydrous sodium sulphate (Na_2SO_4), and dried using nitrogen at 45°C. Further, 0.5 ml 0.5 N KOH in methanol was added to the sample (about 10 mg), and heated at 85°C. Next, 1 ml 12% BF3 in methanol was added, and the sample was again heated at 85°C. After cooling in room temperature, 1 ml hexane and 5 ml saturated solution of NaCl were added. Fatty acid methyl esters profile in one µl samples at the split ratio of 10 : 1 were separated by gas chromatography on a TRACE GC ULTRA gas chromatograph, equipped with 30 m capillary column SUPELCOWAX 10 of 0.25 mm inner diameter and coating thickness of 0.25 µm (30 m × 0.25 mm × 0.25 um). Operating conditions were as follows: helium was used as the carrier gas, flow of 1 ml/min, split flow of 10 ml/min, injector temperature of 220°C, detector temperature of 250°C, and initial column temperature of 160°C.

The atherogenic index (AI) was calculated as (C12:0 + 4 × C14:0 + C16:0)/(MUFA + PUFA), whereas the thrombogenicity index (TI), as (C14:0 + C16:0 + C18:0)/(0.5 × MUFA+

0.5 × n-6PUFA + 3 × n-3PUFA + (n-3 PUFA/n-6 PUFA) (Ulbricht and Southgate 1991).

Molecular analysis

DNA fragments of the MC4R and the LEP genes were amplified from Swallow-Belly Mangalitsa and Moravka animals showing the highest, and the lowest intramuscular fat values. Genomic DNA was isolated from the meat by Genomic Wizard Purification Kit (Promega), according to the described protocol. Primers for amplification of the MC4R and the LEP exons were designed using Primer3 (Table 2). PCR was performed using MJ Mini Thermal Cycler (BioRad) in 25 µl reaction volume (5 µl Colorless GoTaq Flexi Buffer; 2 µl MgCl2, 25 mM; 1 µl dNTP (0,2 mM each); 1 µl of primers (forward + reverse); 0,25 µl GoTaq® G2 Hot Start Polymerase (5u/µl); 1 µl template DNA and Nuclease-Free Water

TABLE 2. Primers and PCR conditions

Fragment	Primer sequences (5'–3')	Size (bp)	Ta (ºC)
Lep_1_for	GTTTCCAGGCCCCAGAAG	200	58
Lep_1_rev	GTCTCCCAGGCCTTCCCTAC		
Lep_2_for	CTGCACAGCAGTCTGTCTCC	379	59
Lep_2_rev	CCTTCAAGGCTTCAGCAG		
MC4R_1-2_for	ATGAACTCAACCCATCACCA	189	58
MC4R_1-2_rev	CAGAGTCACAAACACCTCAGGA		
MC4R_3_for	TCATCTGTAGCCTGGCTGTG	907	62
MC4R_3_rev	CAGAGACTGAGCAGAATCACG		
TaqI_RFLP_for*	TACCCTGACCATCTTGATTG	226	56
TaqI_RFLP_rev*	ATAGCAACAGATGATCTCTTTG		

* according to Kim et al. (2000)

to total volume of 25 µl). The PCR products were purified using Exo_SAP, and then sequenced using BigDye Terminator Sequencing Kit (Applied Biosystems) on ABI 3130xl sequencing system (Applied Biosystems). All sequences were visually inspected, edited, and assembled using FinchTV and BLAST tools, after which the CodonCode Aligner (http://www.codoncode.com/aligner) was applied in order to align the sequences. The SNPs were identified by aligning the obtained sequences with the reference sequence of Sscrofa11.1 – NC_010443.5 for the MC4R and NC_010460.4 for the LEP. All animals were genotyped for MC4R G- > A substitution in 298 codon by means of the PCR-RFLP method, described by Kim et al. (2000).

Statistical analysis

Associations were investigated in the analysis of variance using STATISTICA software 13.1; specifically, the following models:

$$Yijk = \mu + Bi + Sj + (B \times S)ij + \beta Mijk + + eijk$$

$$Yijk = \mu + Gi + Sj + (G \times S)ij + \beta Mijk + + eijk$$

where:

Yijk – studied traits;
μ – overall mean value of the given trait;
Bi – fixed effect of the *i*-th breed (*i* = 1,2,3);
Gi – fixed effect of the *i*-th genotype (*i* = 1,2,3);
Sj – fixed effect of the *j*-th gender (*j* = 1,2);
$(B \times S)ij$ – the correlation between breed and gender;
$(G \times S)ij$ – the correlation between genotype and gender;
$\beta Mijk$ – linear regression of slaughter weight;
eijk – residual effect. The significance of the differences was determined by applying the Tukey-Kramer test.

RESULTS

In silico analysis of the obtained sequences revealed one mutation within the MC4R gene – c.892A>G (Asp298Asn) substitution. The PCR-RFLP bands for both breeds – Swallow-Belly Mangalitsa and Moravka – are presented in Figure 1. In Table 3, allele frequency and genotypes are shown. For Swallow-Belly Mangalitsa, the frequency of the G allele was 97.7%, while for Moravka, it was 79.2%. The GG genotypes frequency ranged from 58.3% for Moravka to 95.4% for Mangalitsa. In Table 4, the chemical composition and shear force analysis results are presented. The highest intramuscular fat content (%) was found in Moravka meat (10.77 ±4.28), as compared to Swallow-Belly Mangalitsa (9.05 ±3.04), and Moravka × Swallow-Belly Mangalitsa (6.76 ±1.31). Total collagen content (%) was the highest in Moravka (0.44 ±0.15), as compared to Swallow-Belly Mangalitsa and Moravka × Swallow-Belly Mangalitsa (0.39 ±0.42 and 0.34 ±0.37, respectively). The highest shear force value was recorded for Moravka × Swallow-Belly Mangalitsa (83.81 ±11.95), as compared to Moravka and Swallow-Belly Mangalitsa (80.52 ±14.38, and 77.19 ±12.85, respectively). In Table 5, the association analysis between identified

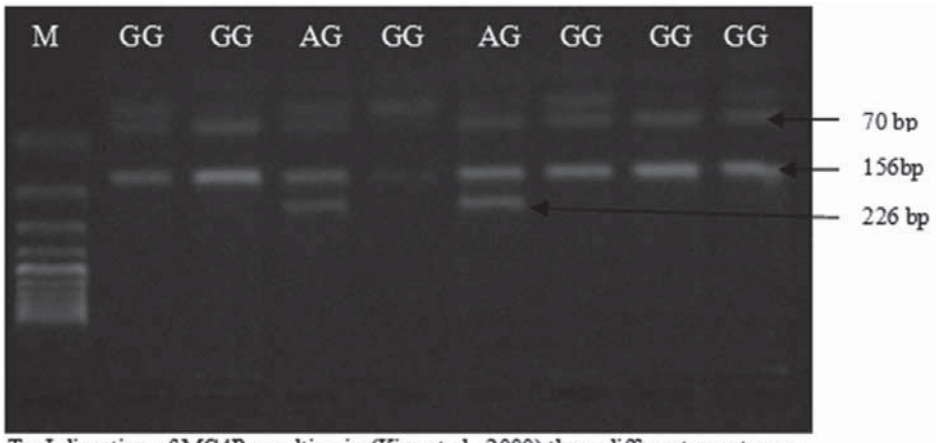

FIGURE 1. TaqI digestion of c.892G>A (D298N) polymorphism within MC4R

TABLE 3. Allele and genotypes frequencies from Moravka and Swallow-Belly Mangalitsa

Polymorphism	Breed	*N*	Allele frequency (%)		Genotypes frequency (%)		
			G	A	GG	AG	AA
c.892G>A	Swallow-Belly Mangalitsa (SBM)	22	97.7	2.3	21	1	0
	Moravka (MO)	12	79.2	20.8	7	5	0

TABLE 4. Chemical composition and shear force analysis of meat from Moravka, Swallow-Belly Mangalitsa and Moravka × Swallow-Belly Mangalitsa crossbred

Traits	Moravka (MO)	Swallow-Belly Mangalitsa (SBM)	Moravka × Swallow-Belly Mangalitsa (MO × SBM)
Slaughter weight (kg)	100.48[a] ±18.53	88.31[b] ±13.95	98.86 ±19.69
Cooking loss (%)	26.69 ±4.85	27.98 ±4.41	27.69 ±2.9
Water (%)	66.64 ±3.85	67.75 ±2.54	69.06 ±1.34
Ash (%)	0.99 ±0.19	0.98 ±0.18	1.01 ±0.12
Carbohydrate (%)	0.32 ±0.15	0.37 ±0.15	0.26 ±0.13
Protein (%)	21.27 ±2.09	21.85 ±1.43	22.97 ±1.44
Intramuscular fat (%)	10.77[a] ±4.28	9.05 ±3.04	6.76[b] ±1.31
Total collagen (%)	0.44 ±0.15	0.39 ±0.42	0.34 ±0.37
Soluble collagen (%)	0.08 ±0.13	0.04 ±0.03	0.08 ±0.09
Unsoluble collagen (%)	0.36 ±0.15	0.35 ±0.41	0.26 ±0.34
Shear Force (N)	80.52 ±14.38	77.19 ±12.85	83.81 ±11.95
Hardness (N)	96.95 ±15.74	102.67 ±19.45	105.54 ±12.19
Springiness	0.52 ±0.08	0.49a ±0.08	0.57b ±0.07
Cohesiveness	0.47 ±0.08	0.45 ±0.06	0.46 ±0.05
Gumminess	48.08 ±16.39	43.56 ±10.11	39.07 ±17.35
Chewiness	25.11 ±10.48	21.6 ±6.16	22.1 ±9.89
Resilience	0.19[a] ±0.04	0.16[b] ±0.02	0.17 ±0.02
Cutting Strength (kg)	3.9 ±1.51	4.54 ±1.34	4.6 ±2.13

Means with different letters differ significantly between groups in a observation ($P \leq 0.05$).

genotypes and meat traits is presented. Because in Swallow-Belly Mangalitsa we identified only one genotype (GA), it was excluded from the analysis. The latter had shown no statistical differences between genotypes in Moravka breed. In Table 6, fatty acids profiles for the analysed breeds are presented. The levels of CLA (conjucted linolic acid) were the highest in Moravka breed (0.079 ±0.01), as compared to Swallow-Belly Mangalitsa (0.072 ±0.007), and to crossbred animals (0.078 ±0.005). Level of polyunsaturated fatty acids was the highest in meat from Swallow-Belly Mangalitsa, as compared to Moravka and to crossbred animals. Saturated fatty acids (41.412 ±0.731) level was the highest in crossbred animals, as compared to Moravka (40.404 ±0.936), and to Swallow-Belly Mangalitsa (40.366 ±0.366), while unsaturated fatty acids level was the highest in Swallow-Belly Mangalitsa (59.604 ±0.361), as compared to Moravka (59.565 ±0.931), and to crossbred animals (58.559 ±0.730). In Table 7, association analysis for fatty acids profile between identified genotypes in Moravka breed was presented, showing lack of statistical differences.

TABLE 5. Association between c.892G > A mutation and chemical composition and shear force analysis of Moravka breed

Traits	Moravka (MO)	
	GG	GA
Slaughter weight (kg)	94.17 ±15.97	106.88 ±20.77
Cooking loss (%)	24.11 ±3.59	25.54 ±1.85
Water (%)	65.58 ±1.91	66.85 ±3.19
Ash (%)	0.96 ±0.01	0.95 ±0.2
Carbohydrate (%)	0.3 ±0.14	0.41 ±0.3
Protein (%)	22.7 ±0.94	20.55 ±1.97
Intramuscular fat (%)	10.45 ±1.86	11.24 ±3.39
Total collagen (%)	0.45 ±0.29	0.48 ±0.13
Soluble collagen (%)	0.23 ±0.29	0.07 ±0.1
Unsoluble collagen (%)	0.22 ±0.2	0.4 ±0.1
Shear Force (N)	72.46 ±7.56	70.63 ±12.82
Hardness (N)	93.35 ±6.44	83.01 ±11.54
Springiness	0.49 ±0.04	0.48 ±0.08
Cohesiveness	0.44 ±0.09	0.48 ±0.11
Gumminess	40.58 ±6.03	40.7 ±14.08
Chewiness	20.06 ±4.5	19.77 ±8.98
Resilience	0.18 ±0.01	0.2 ±0.06
Cutting Strength (kg)	3.91 ±2.64	4.98 ±1.27

TABLE 6. Fatty acids profile (% of total fatty acids) of meat from Moravka. Swallow-Belly Mangalitsa and Moravka × Swallow-Belly Mangalitsa crossbred

Traits	Moravka (MO)	Swallow-Belly Mangalitsa (SBM)	Moravka × Swallow--Belly Mangalitsa (MO × SBM)
10 : 0	0.132 ±0.009	0.137 ±0.012	0.129 ±0.006
12 : 0	0.097 ±0.007	0.1^{a} ±0.004	0.093^{b} ±0.004
14 : 0	1.543^{a} ±0.069	1.506^{b} ±0.034	1.501 ±0.042
14 : 1	0.037^{a} ±0.003	0.039^{b} ±0.004	0.032^{c} ±0.002
15 : 0	0.044 ±0.015	0.043 ±0.006	0.047 ±0.011
16 : 0	27.378 ±0.548	27.293 ±0.345	27.377 ±0.411
16 : 1 n-9	0.319 ±0.026	0.311 ±0.025	0.298 ±0.021
16 : 1 n-7	4.433^{a} ±0.326	4.6^{a} ±0.307	3.977^{b} ±0.133
17 : 0	0.184 ±0.078	0.165^{a} ±0.028	0.219^{b} ±0.063
17 : 1	0.26 ±0.083	0.218^{a} ±0.043	0.301^{b} ±0.083
18 : 0	11.02^{a} ±0.455	10.983^{a} ±0.316	11.88^{b} ±0.525
18 : 1 n-9	41.9^{a} ±0.971	40.227^{b} ±0.821	42.33^{a} ±0.738
18 : 1 n-7	5.074^{a} ±0.278	5.266^{b} ±0.297	4.631^{c} ±0.198
18 : 2 n-6	5.262^{a} ±0.950	6.325^{b} ±0.562	4.9^{a} ±0.547

TABLE 6 – cont.

Traits	Moravka (MO)	Swallow-Belly Mangalitsa (SBM)	Moravka × Swallow--Belly Mangalitsa (MO × SBM)
18 : 3 n-6	0.065 ±0.121	0.039 ±0.004	0.043 ±0.005
18 : 3 n-3	0.176 ±0.068	0.203[a] ±0.012	0.166[b] ±0.034
CLA	0.079[a] ±0.010	0.072[b] ±0.007	0.078 ±0.005
20 : 0	0.135[a] ±0.014	0.140[a] ±0.010	0.165[b] ±0.012
20 : 1	0.614[a] ±0.054	0.518b ±0.083	0.633a ±0.041
20 : 2	0.168 ±0.033	0.166 ±0.012	0.158±0.015
20 : 3 n-6	0.082[a] ±0.018	0.115[b] ±0.012	0.088[a] ±0.006
20 : 4 n-6	0.735[a] ±0.132	1.175[b] ±0.228	0.683[a] ±0.110
20 : 5 n-3	0.022[a] ±0.004	0.035[b] ±0.006	0.025[a] ±0.007
22 : 4 n-6	0.095[a] ±0.017	0.117[b] ±0.02	0.098[a] ±0.011
22 : 5 n-3	0.077[a] ±0.024	0.126[b] ±0.017	0.083[a] ±0.012
22 : 6 n-3	0.037[a] ±0.01	0.052[b] ±0.012	0.031[a] ±0.008
SFA	40.404[a] ±0.936	40.366[a] ±0.366	41.412[b] ±0.731
UFA	59.565[a] ±0.931	59.604[a] ±0.361	58.559[b] ±0.730
n6	6.197[a] ±1.009	7.771[b] ±0.728	5.813[a] ±0.548
n3	0.311[a] ±0.087	0.416[b] ±0.038	0.305[a] ±0.044
n6/n3	20.674[a] ±3.842	18.72[b] ±1.234	19.176 ±1.241
UFA/SFA	1.475[a] ±0.058	1.477[a] ±0.022	0.129[b] ±0.006
EFA	5.504[a] ±1.036	6.567[b] ±0.569	5.11[a] ±0.569
OFA	28.921 ±0.546	28.799 ±0.34	28.878 ±0.34
DFA	70.585 ±0.669	70.586 ±0.342	70.439 ±0.342
MUFA	52.637[a] ±1.426	51.178[b] ±0.606	52.205[a] ±0.606
PUFA	6.72[a] ±1.153	8.353[b] ±0.763	6.276[a] ±0.763
AI	0.567[a] ±0.017	0.561[a] ±0.008	0.573[b] ±0.008
A-SFA	29.018 ±0.547	28.899 ±0.34	28.971 ±0.34
T-SFA	39.94[a] ±0.823	39.781[a] ±0.356	40.758[b] ±0.356
TI	1.313[a] ±0.043	1.293[a] ±0.021	1.36[b] ±0.021
Δ9-desaturase index	0.542[a] ±0.01	0.535[b] ±0.003	0.537 ±0.003

SFA – saturated fatty acids; UFA – unsaturated fatty acids; OFA – hypercholesterolemic acids (C14 : 0 + C16 : 0); DFA – neutral and hypocholesterolemic acids (C18:0 + UFA);

AI index – (C12:0 + 4 × C14:0 + C16:0) / [(MUFA + ΣSPUFA (n-6) + (n-3)] (Ulbricht et al. 1991);

A-SFA – the sum of C12:0.C14:0 and C16:0;

T-SFA – the sum of C14:0. C16:0 and C18:0;

TI – (C14:0 + C16:0 + C18:0) / (0.5 × MUFA + 0.5 × n-6-PUFA + 3 × n-3PUFA + (n-3PUFA/n-6PUFA)) (Ulbricht et al. 1991).

Δ9-desaturase index = (C16:1 + c9C18:1 + c11C18:1) / (C16:1 + c9C18:1 + c11C18:1 + C14:0 + C16:0 + C18:0) (Smith et al. 2002)

Means with different letters differ significantly between groups in a observation ($P \leq 0.05$).

TABLE 7. Association between c.892G > A mutation and Fatty acids profile (% of total fatty acids) of meat from Moravka breed

Traits	Moravka	
	GG	GA
10 : 0	0.138 ±0.009	0.130 ±0.005
12 : 0	0.099 ±0.008	0.098 ±0.007
14 : 0	1.554 ±0.063	1.572 ±0.048
14 : 1	0.037 ±0.003	0.036 ±0.003
15 : 0	0.04 ±0.011	0.038 ±0.007
16 : 0	27.36 ±0.534	27.373 ±0.521
16 : 1 n-9	0.323 ±0.029	0.31 ±0.01
16 : 1 n-7	4.41 ±0.386	4.419 ±0.185
17 : 0	0.155 ±0.068	0.171 ±0.058
17 : 1	0.202 ±0.033	0.288 ±0.065
18 : 0	10.861 ±0.565	10.942 ±0.34
18 : 1 n-9	42.312 ±0.343	42.003 ±0.268
18 : 1 n-7	5.332 ±0.106	5.041 ±0.21
18 : 2 n-6	5.026 ±0.386	5.259 ±0.476
18 : 3 n-6	0.036 ±0.012	0.042 ±0.006
18 : 3 n-3	0.129 ±0.021	0.173 ±0.039
CLA	0.079 ±0.004	0.083 ±0.006
20 : 0	0.134 ±0.015	0.126 ±0.003
20 : 1	0.628 ±0.08	0.605 ±0.024
20 : 2	0.164 ±0.02	0.163 ±0.02
20 : 3 n-6	0.067 ±0.012	0.082 ±0.015
20 : 4 n-6	0.658 ±0.051	0.784 ±0.142
20 : 5 n-3	0.024 ±0.003	0.02 ±0.002
22 : 4 n-6	0.083 ±0.006	0.097 ±0.015
22 : 5 n-3	0.068 ±0.012	0.08 ±0.02
22 : 6 n-3	0.031 ±0.008	0.04 ±0.003
SFA	40.009 ±0.702	40.449 ±0.432
UFA	59.944 ±0.677	59.522 ±0.431
n6	5.87 ±0.453	6.263 ±0.475
n3	0.252 ±0.038	0.312 ±0.057
n6/n3	23.614 ±4.072	20.482 ±3.365
UFA/SFA	1.499 ±0.043	1.472 ±0.026
EFA	5.192 ±0.394	5.473 ±0.47

TABLE 7 – cont.

Traits	Moravka	
	GG	GA
OFA	28.914 ±0.483	28.944 ±0.53
DFA	70.805 ±0.195	70.465 ±0.51
MUFA	53.245 ±0.636	52.701 ±0.09
PUFA	6.287 ±0.446	6.738 ±0.52
AI	0.566 ±0.015	0.568 ±0.01
A-SFA	29.014 ±0.476	29.043 ±0.53
T-SFA	39.776 ±0.920	39.887 ±0.46
TI	1.311 ±0.053	1.309 ±0.03
Δ9-desaturase index	0.547 ±0.008	0.543 ±0.01

SFA – saturated fatty acids; UFA – unsaturated fatty acids; OFA – hypercholesterolemic acids (C14:0 + C16:0). DFA – neutral and hypocholesterolemic acids (C18:0 + UFA);

AI index – (C12:0 + 4 × C14:0 + C16:0) / [(MUFA + ΣSPUFA (n-6) + (n-3)] (Ulbricht et al.. 1991);

A-SFA – the sum of C12:0. C14:0 and C16:0;

T-SFA – the sum of C14:0. C16:0 and C18:0;

TI – (C14:0 + C16:0 + C18:0) / (0.5 × MUFA + 0.5 × n-6- PUFA + 3 × n-3PUFA + (n-3PUFA/ /n-6PUFA)) (Ulbricht et al. 1991).

Δ9-desaturase index = (C16:1 + c9C18:1 + c11C18:1)/(C16:1 + c9C18:1 + c11C18:1 + C14:0 + C16:0 + C18:0) (Smith et al. 2002).

DISCUSSION

Fatness is an important trait, influencing meat quality and its technological values. Pork is an important source of meat for humans, accounting for more than half the world's meat consumption. Moreover, consumers demand the highest quality meat, and high quality meat products, in the constantly changing pork market (Moeller et al. 2010). Nowadays, consumers show preference for sustainable pork chains, whereas traditional products from local pig breeds have proven themselves as high quality products (Pugliese and Sirtori 2012, Pugliese et al. 2013). Reports by Petrović et al. 2010 showed that intramuscular fat (IMF) content (%) in Moravka is lower compared to Mangalitsa (6.74 and 13.24, respectively); also, cholesterol level (mg/100g) is lower in Moravka compared to Mangalitsa (42.14 and 61.82, respectively). Our results showed that intramuscular fat content was the highest in Moravka (10.77 ±4.28), as compared to Swallow-Belly Mangalitsa (9.05 ±3.04), and Moravka × Swallow-Belly Mangalitsa crossbreed (6.76 ±1.31). Intramuscular fat is generally associated with higher quality of nutrition, therefore, it is of much significance to increasing intramuscular fat content of pork through feeding and genetic selection. Feeding has several disadvantages, because particular feed-

ing regimes need to be developed for various breeds. The most promising way is that of the genetic selection, however, the existence of numerous quantitative traits loci (QTL) may be problematic (Font-i-Furnols et al. 2012). Among the most analysed candidate genes for IMF value were: leptin (LEP) and Melanocortin 4 Receptor (MC4R). Kim et al. 2000 analysed c.892G>A (D298N) polymorphism, and they found association with back-fat and growth rate. Fontanesi et al. (2013) found association with average daily gain (ADG) and the feed:gain ratio (FGR) with c.892A. In our results, we did not find statistical significance between the genotypes, but we found that for slaughter weight, the GA genotypes were heavier compared to GG, similar to the IMF value (Table 3).

According to the current state of knowledge, n-6:n-3 PUFA (polyunsaturated fatty acids) ratio should be limited to 4–5 : 1 (Scollan et al. 2006). In our study, the ratio was higher than those values. Ulbricht and Southgate (1991) reported indicators of fat quality, including the atherogenicity index (AI), which likely reflects the risk of cardiovascular disease (CVD). It defines the proportion of SFA (myristic and palmitic acid) to UFA (PUFA + MUFA), indicating a negative role of C14:0, and an adverse effect of UFA in human nutrition. The lower the AI and TI index values, the healthier the food. In our study, statistically significant differences were found between pure breeds and crossbred pigs, where crossbred pigs had highest values of AI and TI indexes (0.573 ±0.008 and 1.36 ±0.021, respectively). Ulbricht and Southgate (1991) suggested that the AI index should be lower than 0.5. The levels of 20:3n-6; 20:4n-6; 20:5n-3; 22:4n-6; 22:5n-3; 22:6n-3 were statistically higher in meat from Mangalitsa compared to Moravka. Galián et al. (2008) pointed out that the PUFA levels should not be higher than 12–14% in meat destined to become processed products, and this was confirmed in our study. The polyunsaturated fatty acids and monounsaturated fatty acids play a role in decreasing the blood LDL-cholesterol concentration, by increasing the hepatic LDL receptor activity (Rudel et al. 1995). Cameron et al. (2000) showed that C18:2, C20:4 and C22:6 polyunsaturated fatty acids had a positive correlation with the flavor of meat.

Compared to Zlotnicka Spotted and Pulawska, the content of protein is similar (about 21–22%) (Florowski et al. 2006), however, intramuscular fat content in Polish autochthonous pig breeds is between 2.5% (Pulawska) and 3.1% (Zlotnicka Spotted), while for Moravka, it is 10.77 ±4.28, and for Swallow-Belly Mangalitsa, it is 9.05 ±3.04. Differences can also be noticed in terms of shear force – its range is between 80.52 ±14.38 for Moravka, and between 83.81 ±11.95 for crossbreed pigs, while for Zlotnicka Spotted and for Pulawska, it is 18.3 ±4.4, and 31.7 ±10.6, respectively.

In summary, results from our study showed that Moravka and Swallow-Belly Mangalitsa have high quality meat, which can be used in the manufacturing of traditional products. Meat from Moravka and Mangalitsa are used for production of cajna sausage and sremska sausage, petrovská sausage and Serbian pork ham. Those products have high acceptability in Balkan region, which show high quality of those products.

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Streszczenie: *Parametry jakości mięsa i profil kwasów tłuszczowych świń rasy mangalica jaskółcza, morawka oraz ich mieszańców.* Rasy autochtoniczne wiążą się z kulturą i tradycją danych regionów. Stanowią one bardzo ważny element krajobrazu, jak i są źródłem produktów wysokiej jakości. Celem pracy była analiza parametrów fizykochemicznych, składu chemicznego oraz profilu kwasów tłuszczowych mięśnia najdłuższego grzbietu świń rasy Moravka, Swallow-Belly Mangalitsa i ich mieszańców. Ponadto zsekwencjonowano fragmenty kodujące geny leptyny (LEP) oraz receptora 4 melanokortyny (MC4R). W obrębie genu MC4R zidentyfikowano mutację c.892A>G (Asp298Asn). Analiza statystyczna nie wykazała różnic między genotypami. Pomiędzy rasami statystyczne różnice wystąpiły dla zawartości tłuszczu (%) między rasą Moravka a mieszańcami (10,77 ±4,28 i 6,76 ±1,31). Rasa Swallow-Belly Mangalitsa charakteryzowała się statystycznie największą zawartością n-3 i n-6 PUFA (7,771 ±0,728 i 0,416 ±0,038). Poziom CLA był najwyższy u świń rasy Moravka, a najniższy u świń rasy Swallow-Belly Mangalitsa (0,079 ±0,010, and 0,072 ±0,007). Wyniki naszych badań potwierdzają, wysokie parametry jakościowe mięsa pochodzącego od świń rasy Moravka jak i Swallow-Belly Mangalitsa.

Słowa kluczowe: świnie, mangalica jaskółcza, morawka, cechy jakości mięsa, profil kwasów tłuszczowych, *LEP*, *MC4R*

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Authors' addresses:
Łukasz Migdał
Katedra Genetyki i Metod Doskonalenia Zwierząt
Wydział Hodowli i Biologii Zwierząt
Uniwersytet Rolniczy im. H. Kołłątaja w Krakowie
al. Mickiewicza 24/28, 30-059 Kraków
Poland
e-mail: lukasz.migdal@urk.edu.pl