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Research

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**Comparison of Growth Performance and Meat Quality Traits of Commercial Cross-bred Pig
versus Large Black Pig Breeds**

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Abstract

Background: The meat quality of different pig breeds is associated with their different muscle tissue physiological processes, which involves a large variety of genes related with muscle fat and energy metabolism. Understanding the differences of biological processes of muscle after slaughter is helpful to reveal the meat quality development of different breeds. Therefore, 8 domestic Large Black pigs (BP), a domestic breed with high fat contents in meat, and 7 Cross-bred commercial pig (CP), which has a high feed efficiency with high lean meat, was used to investigate the differences of their meat quality and genotype.

Results: The average daily gain (ADG) and hot carcass weight (HCW) of CP were higher than BP, but the back-fat thickness of BP was higher than CP ($P < 0.05$). The CP had higher a* but lower h value than BP ($P < 0.05$). The metmyoglobin (MMb) percentage of CP was higher ($P < 0.05$) than BP. The fat content and oxygen consumption of *longissimus dorsi* (LD) muscle in BP were higher ($P < 0.05$) than CP. BP had higher SFA and MUFA content, but CP had higher PUFA content ($P < 0.05$). The RNA-seq was applied to compare the genome differences between the two pig breeds. The RNA-seq data highlighted 201 genes differentially expressed between breeds ($P < 0.05$), with 75 up-regulated and 126 down-regulated genes in BP compared with CP. The real-time PCR was used to validate the results of RNA-seq for 8 genes, and the genes related with lipid and energy metabolism were highly expressed in BP ($P < 0.05$).

Conclusions: Based on the results, BP had superior general meat quality to CP, while the growth performance of CP was better, and the genotype differences between these two breeds may cause the meat quality and growth performance variance.

Keywords

Commercial cross-bred pigs; British Large Black pigs; Meat quality; Breeds; Intramuscular fat; RNA-seq; Gene expression

Background

Increasing carcass weight and leanness of pigs have been a strong emphasis on production efficiency in swine breeding programs for many years. The researchers concentrated on improving pork production such as growth rate and feed conversion ratio, and they also aimed at decreasing carcass

fat content and backfat thickness. However, pork quality has received a prime focus on pig production as consumers demand better meat quality, for example, tenderness, marbling, color, and water holding capacity (WHC) [1]. E Dransfield, T Ngapo, NA Nielsen, L Bredahl, P-O Sjöden, M Magnusson, M Campo and G Nute [1]. The interactive effects of pig breeds, environmental situations, pre-harvest management and post-harvest process result in different meat quality [2]. However, the effects of genotype on meat eating quality, generally, are higher than external feed conditions [3]. It was reported that meat quality was also related to postmortem meat metabolic, and oxygen consumption of muscle tissue is one of its phenotype [4]. In addition, a large variety of genes related to both muscle structures and metabolic substances could affect the postmortem physiological developments inside the muscle cells. Comparing the transcriptome expression profile differences between different breeds could help us to understand the principles of genes related with meat quality and muscle biological processes.

Commercial cross-bred pigs (CP) are widely used in the world pig industry for its great feed efficiency and higher average daily gain (ADG), with high productivity of lean meat and less fat content. In contrast, the British Large Black pigs (BP) are British domestic pigs with long and deep-bodied and are well known for its high-fat contents and high meat quality characteristics [5, 6].

The aim of this experiment was to investigate the carcass, meat quality traits of British Large Black Pigs (BP) and Commercial Cross-bred pigs (CP) in relation to their transcriptome profiles, and consequently clarify the phenotypic and genotypic differences between these two breeds. The outcome of the study provided new sights into the adoption of British Large Black breed in pig industry. In order to investigate the genome organization's complexity and the transcriptional landscape of tissues and cells between different breeds, comparative transcriptome analysis is freshly used [7]. However, in the past, general gene expression was determined by microarray hybridization technology [8]. Recently, sequencing technology offers more benefits than microarrays, because of it rapidly advance. For instances, RNA-seq concentrates more on the reconstruction of the entire transcriptome [9], and provides a complete comprehension of genotype items [10]. On the base of RNA-seq, functional analysis of Gene Ontology (GO) biological process (BP) terms can be used to highlight the genes association and the networks of relevant biological

pathways. The real-time PCR is a verification of RNA-seq data. Hence, RNA-seq and real-time PCR could be useful tools to investigate the reasons attributed to carcass and meat quality traits between BP and CP.

Material and methods

Animals

Before the initiation of research, the University of Arkansas's Institutional Animal Care and Use Committee approved all procedures of the experiment involving animals during the study. Eight British Large Black pigs and seven commercial Cross-bred pigs were allocated to BP group and CP group, and their initial mean body weights were tabulated (23.31 ± 1.93 kg for BP group and 18.82 ± 1.41 kg for CP group). They were fed *ad libitum* and kept individually in digestibility pens for 101 days. The final body weight of CP groups was determined at the end of 101-day experiment. However, due to low growth performance of the BP, the average body weight of BP group was 115.4 kg, lower than the average market live weight which was 127.9 kg (<https://www.pork.org/facts/stats/consumption-and-expenditures/typical-market-pig-today>). The BP group was kept for 108 days. The difference value between initial and final body weight was used to calculate ADG. Animals were off feed 12 h prior to slaughter, with access to water. Then, they were rendered unconscious by a nonpenetration stunning method.

Carcass characteristics and sampling

Immediately after stunning and completion of exsanguination, hot carcass weight (HCW) and backfat thickness (midline, between the 4th and 5th lumbar vertebra level) were determined. Then, muscles from *longissimus dorsi* (LD) of left carcass was removed. One piece of samples was stored at -80°C for RNA isolation process. The carcasses were chilled at -9°C for two hours and then kept at 4°C for 24h. Another piece from LD muscle was obtained (between the 12th and 13th rib) and transported under refrigeration until being processed for meat quality. The muscles were cut into small pieces, vacuum-packed and stored at -20°C before analyzing the fatty acid profile.

Meat quality

The intramuscular fat (IMF) content of *longissimus dorsi* muscle was determined by using Soxhlet

apparatus to extract ether without previous acid hydrolysis [11]. A 2 cm 100g thick slice cut from LD muscle was placed into a polypropylene bag then stored in a vacuum package for 24 h at 4°C, and the weight difference of samples were regarded as drip loss showed as percentage [12]. In order to ensure stable data of color measurements, sample were bloomed for 20 min before experiment. The L, a*, and b* color values were determined by a CR-400 Chroma Meter. Then, the hue angle ($h = \arctan(b^*/a^*)$) and chroma ($C^* = ((a^*)^2 + (b^*)^2)^{0.5}$) was calculated by the L, a*, and b* values.

Fatty acid composition

The fatty acid composition of LD muscle IMF was determined by fat extract [13]. Ten grams minced meat were homogenized at 3000 rpm for 1.5 min by UltraTurrax using 0.003% butylhydroxytoluene (BHT) with 200 ml Folch solution (chloroform-methanol mix 2:1). After paper-filtering (Whatman No. 1) the homogenized liquid, Folch solution (50ml) was added. After filtered, the solution was poured out into a decantation infundibulum mixed with 8% sodium chloride (80ml) for 24 h. The solvent, collected from lipidic phase, was evaporated. After evaporation, the fatty acids composition was analysis using a gas chromatography (Agilent 6890 N Network GC System). As a carrier gas, helium was used at a division ratio of 1:50 with a 3.2 ml per minute flow rate. The undecanoic acid methyl ester was used as an internal standard to quantify the methyl esters of fatty acids.

RNA extraction and cDNA Synthesis

The total RNA of *longissimus dorsi* muscle was extracted by TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The total RNA, extracted from the muscle, was then treated with DNase I (Promega, Madison, WI, USA) to eliminate the contamination of genomic DNA, following the directions of manufacturer. The integrity of total RNA was assessed by NanoDrop (Agilent Technologies, CA, USA). The decontaminated RNA samples were performed to reverse-transcription using Takara PrimeScript™ RT reagent Kit, and then prepared for cDNA synthesis according to the instructions of Takara PrimeScript™ RT.

Real-time PCR

According to the recorded sequences showed in GenBank, the primers of *SLC26A7*, *TKTL2*, *ACBD7*, *THRSP*, *SLPI*, *FADS1*, *ACSL6*, *FOS* and *GAPDH* were designed using Oligo 6.0 Software (Table 3).

The cDNA was used to perform Real-time PCR in order to obtain the expression level of *SLC26A7*, *TKTL2*, *ACBD7*, *THRSP*, *SLPI*, *FADS1*, *ACSL6*, *FOS*. Real time-PCR was performed using 15 µL reaction system: 7.5 µL 2 × Real Master Mix; 0.75 µL upstream and 0.75 downstream primer (10 pmol/L); 3 µL cDNA; and 3 µL water. The reaction liquid was added on iCycler IQ⁵ (Bio-Rad, USA). The reactive conditions and primers were presented in Table 3. Relative expression levels were normalized to GAPDH gene and expressed as fold change [14]

RNA sequencing and functional analysis

The RNA-seq data were aligned, using the STAR aligner, to the most current pig genome (sus scrofa 11.1_v91). Then, the Edge R package in SeqMonk (v.1.37.1) was used for differential gene expression analysis. Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to implement the enrichment analysis for specific GO terms [15, 16]. The gene lists were uploaded using the ENSEMBL gene ID (<http://www.ensembl.org>).

Oxygen consumption and myoglobin calculation

Approximately 20 mg of tissue from *longissimus dorsi* muscle from each sample was put into a respiration buffer (1.1 mM sodium pyruvate, 25 mM glucose in PBS and 2% BSA). The Oxygen consumption rate (OCR) of every sample was measured by an Orion Dissolved Oxygen platform (Scientific) about 25 minutes, repeat 3 times. A full reflectance spectral analysis was also taken on each muscle and wavelength ratios were used to calculate relative deoxymyoglobin (474 nm:525 nm), oxymyoglobin (610 nm : 525 nm), and metmyoglobin (572nm : 525nm) concentrations [17].

Statistical analysis

All the data analysis was performed by SPSS version 19.0 software (SPSS Inc., Chicago, IL). The variance between these two pig breeds was carried out using *t*-test of independent samples. The results were given as means and SD in the text, and the differences was considered significant when $P < 0.05$.

Results and discussion

Growth performance and carcass measurements

The growth performances in terms of initial and final body weight and the quality of carcass are presented in Table 1. The initial body weights of CP and BP groups showed no significant differences ($P > 0.05$). However, CP group had higher final body weight ($P < 0.05$), ADG ($P < 0.05$) and hot carcass weight ($P < 0.01$) compared with BP group. According to R Bessa, R Hughes, E Jeronimo, O Moreira, J Prates and OJJoas Doran [18], the fat deposition of the domestic pig is higher than the crossbreed pig. Correspondingly, the BP group showed a strong fat deposition ability with a significantly higher ($P < 0.01$) back fat thickness values compared to CP.

Meat quality

Meat quality traits of CP and BP groups are summarized in Table 2. Drip loss is one of the important characteristics of water holding capacity (WHC). There is no significant difference showed in drip loss between CP and BP ($P > 0.05$). For the meat color, L^* indicates lightness, a^* is about red/green coordinate, b^* shows the yellow/blue coordinate, C^* means chroma, and h is the hue angle [19]. There was no significant difference of L^* , b^* and C^* between these two groups ($P > 0.05$). In contrast, the a^* value of CP is higher ($P < 0.01$) than BP, but the h value of BP is higher ($P < 0.05$) than CP.

The IMF content of these two breeds was significantly different ($P < 0.05$), that BP is higher than CP. However, the different fat content did not affect the drip loss. G Watanabe, M Motoyama, I Nakajima and KJA-Ajoas Sasaki [20] pointed out that pork IMF content was not correlated with drip loss, and our result had the same trend. The fat content of CP is similar to other reports about white pig breeds [21, 22], and the fat deposition of BP is much higher than these commercial pigs. The research compared Iberian pigs and commercial cross-bred pigs showed a strong correlation between IMF and backfat thickness [23]. Our data showed higher IMF and backfat deposition in the British Large Black pigs, which demonstrate the similarity fat deposition pattern in local domestic pigs that is different from commercial cross-bred pigs.

Oxygen consumption

The OCR is positively correlated with mitochondrial concentration [24]. In addition, mitochondria present in the postmortem muscle can remain active and impact on the meat color through oxygen

consumption [25]. The OCR of BP was higher than in CP ($P < 0.01$; Fig 1), so there should be more mitochondria in LD muscle of BP. It was reported that muscle with more β -red fibers tended to have higher OCR than muscle with more α -white fibers [26]. However, the relationship between oxygen consumption and meat quality needs to be further evaluated to understand color variations between the two breeds. The oxygen consumption also represents the activation of mitochondrial metabolism. A study of the muscle fiber type of Korean native pig reported higher portion of type I muscle fibers than that of the commercial cross-bred pigs [27]. The type I slow twitch fiber has more content of mitochondria for oxidative phosphorylation. This report was consistent with what we found that the local domestic pigs have higher mitochondrial metabolism correlated with the higher content of type I muscle fiber.

Myoglobin calculation

The color of meat after slaughter is primarily affected by myoglobin which is a sarcoplasmic heme protein [28]. This protein can exist as deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb) in fresh meat [25]. Our results showed differences in the percentage of myoglobin between CP and BP breeds (Figure 2). The MMb (metmyoglobin) percentage of CP was higher ($P < 0.05$) than BP, but there were no significant differences between CP and BP in DMb (deoxymyoglobin) and OMb (oxymyoglobin). The chemistry and functions of myoglobin in live muscles and meat may be different, but the functions of myoglobin as the oxygen binder and oxygen deliverer to keep the physiological functions of mitochondria continues normally [29]. In addition, myoglobin is related to red meat color, which is a main meat purchasing factor for consumers [30].

Fatty acid composition

The fatty acid composition of LD muscles from CP and BP breeds are listed in Table 4. There was a significant fatty acid composition difference between CP and BP in most comparisons ($P < 0.05$). The total polyunsaturated fatty acids content of CP was higher ($P < 0.05$) than BP, such as C18:2n-6, C18:3n-3, C20:2, C20:3n-6, C20:4n-6, and C22:5. However, BP had higher total monounsaturated fatty acid content along with higher C:10, C:20, C18:1, and C20:0 contents compared to CP. The overall saturated and unsaturated fatty acids contents of CP and BP had no significant differences ($P > 0.05$).

The fatty acid composition has a strong relationship with the IMF content and backfat thickness [31]. It was reported that intramuscular and backfat would increase the percentage of saturated, especially monounsaturated fatty acids, and the data of our experiment is in agreement with their findings [32]. The fatty acid composition variations in CP and BP are probably attributed to the difference in fat deposition between these two breeds. The SFA level of intramuscular fat in loin has negative correlation with meat sensory quality, such as acid flavor [33]. However, there was no significant differences of SFA between BP and CP. It was reported that monounsaturated fatty acids are the major fatty acids component in the Mediterranean diet which benefits lowering the risk of cardiovascular disease [34], considering the IMF content in BP LD muscle is about 1.6 times higher than CP (10.02 ± 1.20 : 6.24 ± 1.53 , $P < 0.05$, Table 2), which might indicate that BP pork products have higher healthy value.

Differentially expressed gene analysis

High-throughput sequencing is a powerful way to identify the differences in gene expression, which is recently used in the study of different breeds to compared the difference of genes expression related with meat quality [35]. By comparing LD muscle transcriptome differences between BP and CP, we found that there was a total of 384 differentially expressed genes found between these two breeds, in which 201 are highly differentially expressed ($\log_2$ Fold change ≥ 1 or ≤ -1 ; P -value < 0.05). Compared with CP, BP had 75 up-regulated and 126 down-regulated genes (Fig.3.). The functional category of these 201 differentially expressed genes, of which 75 are highly expressed in BP and 126 are highly expressed in CP, were determined by querying associated gene ontologies, and they were classified into biological process, cellular component, and molecular function (Fig.4-6), by using DAVID bioinformatic resources. GO analysis showed the functional enrichment of these differentially expressed genes, and the different genes expression may cause the diversities of carcass characteristics and meat quality between these two breeds. For biological process, 5 highly expressed genes in BP were related with fat cell differentiation (Fig.4). This might have led to a higher IMF content of LD muscle in BP, compared with CP. In molecular function, we found that 6 genes, which were highly expressed in CP, were responsible for oxidoreductase activity, and these genes' higher expression may cause the different OCR of the LD muscle between BP and CP.

Gene expression

In order to verify our RNA-seq expression profile data, we utilized real-time quantitative RT-PCR to determine eight genes related to lipid deposition or metabolism, and the results (shown in Table 7.) validated the transcriptome profiles of RNA-seq. Results are presented as numerical relative gene expression values (using *GAPDH* as a housekeeping calibrator). The expression of lipid and energy metabolic related genes (*TKTL2*, *ACBD7*, *ACSL6*, and *FOS*) were higher in CP than in BP ($P < 0.05$). In addition, CP exhibited higher ($P < 0.05$) mRNA abundance of *THRSP* gene which is related to medium-length fatty acid chains [36]. However, the gene expression of *SLPI* which involved in epithelial immunity was higher in BP ($P < 0.05$) is consistent with the characteristic of stronger environmental tolerance in BP as a local domestic pig breed compared to CP [37]. The gene related to energy metabolism and unsaturated fatty acids composition (*SLC26A7* and *FADS1*) were higher expressed in CP. Pigs of different breeds always have the different condition of metabolism and lipid deposition. *SLC26A7* is strongly related to energy metabolism [38]. *TKTL2* is related with lipid transport and metabolism, and the genetic distance increased from local pig breeds after selective seep caused by the selection of energy-rich pork production [39]. *ACBD7* is one of the gene family related with intracellular lipid-binding proteins [40]. *FADS1* gene is associated with the unsaturated fatty acid composition [41]. It was reported that *ACSL6* contributes to lipid synthesis [42]. G Reiner, L Heinrich, E Müller, H Geldermann and V Dzapo [43] reported that *FOS* is an important gene correlated with skeletal muscle fiber and metabolism. Above all, the differential of gene expression related to lipid and energy metabolism might cause the different fat deposition ability of CP and BP.

Conclusion

In conclusion, the growth performance of CP was higher than BP, but the meat quality traits of BP was better than CP. The difference in intramuscular fat content, oxygen consumption and myoglobin calculation of LD muscle between these two breeds were also high. The RNA-seq and gene expression data proved an efficient sight about the differences of transcriptome profiles and genotype of these two breeds. Comparing BP with CP, 201 significantly differentially expressed genes in LD muscle were identified.

286 **List of Abbreviation**

287	BP	Black pigs
288	CP	Cross-bred commercial pig
289	ADG	Average daily gain
290	HCW	Hot carcass weight
291	MMb	Metmyoglobin
292	DMb	Deoxymyoglobin
293	OMb	Oxymyoglobin
294	LD	Longissimus dorsi
295	SFA	Saturated fatty acid
296	UFA	Unsaturated fatty acid
297	MUFA	Monounsaturated fatty acid
298	PUFA	Polyunsaturated fatty acid
299	WHC	Water holding capacity
300	GO	Gene Ontology
301	PCR	Polymerase chain reaction
302	IMF	Intramuscular fat
303	BHT	Butylhydroxytoluene
304	RNA	Ribose nucleic acid
305	cDNA	Complementary deoxyribonucleic acid
306	SLC26A7	Solute carrier family 26 member 7
307	TKTL2	Transketolase like 2
308	ACBD7	Acyl-CoA binding domain containing 7
309	THRSP	Thyroid hormone responsive
310	SLPI	Secretory leukocyte peptidase inhibitor
311	FADS1	Fatty acid desaturase 1
312	ACSL6	Acyl-CoA synthetase long chain family member 6
313	FOS	Fos proto-oncogene, ap-1 transcription factor subunit
314	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
315	DAVID	Database for annotation, visualization and integrated discovery
316	OCR	Oxygen consumption rate
317		

Declarations:

Ethics approval

the University of Arkansas's Institutional Animal Care and Use Committee approved all procedures of the experiment involving animals during the study.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

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Authors' contributions

YW, SS, JC analyzed the data. YW, SS, YH took measurements and collected samples. KT conducted RNA sequencing and functional analysis. YW, SS wrote the manuscript. All authors read and approved the final manuscript.

Legend:

Table 1. Effect of breeds on the carcass characteristics (CP: Cross-bred commercial pigs, BP: British Large Black pigs)

^a ADG: Average daily gain

^b NS: not significant/(*), (**), (***) Significant $P < .05$, 0.01, 0.001 respectively

Table 2. Effect of breeds on the meat quality of pork (*longissimus dorsi*) (CP: Cross-bred commercial pigs, BP: British Large Black pigs).

^a L*: Lightening; a*: red-green; b*: yellow-blue; C*: bright-dull; h: hue

^b NS: not significant/(*), (**), (***) Significant $P < .05$, 0.01, 0.001 respectively

Fig. 1. Effect of breeds on the oxygen consumption (*longissimus dorsi*) (CP: Cross-bred commercial pigs; BP: British Large Black pigs; NS: not significant/(*), (**), (***) Significant $P < .05$, 0.01, 0.001, respectively)

Fig. 2. Effect of breeds on the myoglobin calculation (*longissimus dorsi*) (CP: Cross-bred commercial pigs, BP: British Large Black pigs; MMb: Metmyoglobin, DMb: Deoxymyoglobin, OMb: Oxymyoglobin; NS: not significant/(*), (**), (***) Significant $P < .05$, 0.01, 0.001, respectively)

Fig. 3. Volcano figure of RNA-seq (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs; Up: up regulated in BP compared with CP; Normal: No significant differences; Down: down regulated in BP compared with CP)

Fig. 4. Gene ontology (GO) analysis of differentially expressed genes highly express in BP (A) and CP (B). The identified differentially expressed genes were classified in to Biological Process. The numbers of genes the GO term is shown above (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs)

Fig. 5. Gene ontology (GO) analysis of differentially expressed genes highly express in BP (A) and

CP (B). The identified differentially expressed genes were classified in to Cellular Component. The numbers of genes the GO term is shown above (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs)

Fig. 6. Gene ontology (GO) analysis of differentially expressed genes highly express in BP (A) and CP (B). The identified differentially expressed genes were classified in to Molecular Function. The numbers of genes the GO term is shown above (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs)

Fig. 7. Effect of breeds on the related gene expression (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs) (*) Significant $P < .05$.

Table 3. Primer information for gene chosen for confirmation of expression using quantitative real-time PCR.

Table 4. Effect of breeds on the fatty acid profile of intramuscular fat (*Longissimus dorsi*) (CP: Cross-bred commercial pigs, BP: British Large Black pigs)

NS: not significant/(*), (**), (***) Significant $P < .05$, 0.01, 0.001, respectively

Table 1.

Parameters	CP		BP		<i>P</i> -Value ^b
	Mean	sd	Mean	sd	
Initial body weight (kg)	18.82	1.41	23.31	1.93	NS
Final body weight (kg)	130.04	8.16	121.17	2.80	*
ADG ^a (kg)	1.10	0.05	0.91	0.01	*
Hot carcass weight (kg)	77.95	9.81	63.63	0.95	**
Back fat thickness (cm)	0.72	0.07	1.42	0.22	***

Table 2.

Parameters	CP		BP		<i>P</i> -Value ^b
	Mean	sd	Mean	sd	
Drip loss (%)	3.38	0.11	3.41	0.17	NS
L ^{*a}	57.78	4.52	60.24	2.43	NS
a ^{*a}	16.72	1.73	14.58	1.09	**
b ^{*a}	14.31	1.68	13.73	0.67	NS
C ^{*a}	22.02	2.28	19.99	1.09	NS
h ^a	40.49	2.13	43.47	1.64	*
<i>Proximal composition:</i>					
Total fat (%)	6.24	1.53	10.02	1.20	*

387 Fig. 1.

388

Fig. 2.

391 Fig. 3.

392

A

B

A Enriched Go Terms (Cellular Component) in BP

B Enriched Go Terms (Cellular Component) in CP

A Enriched Go Terms (Molecular Function) in BP

B Enriched Go Terms (Molecular Function) in CP

401 Table 3.

Gene	Direction	Primer sequence	GenBank
			Accession No.
GAPDH	Forward	5'- TCGGAGTGAACGGATTTGGC -3'	NM_00120659.1
	Reverse	5'- TGACAAGCTTCCCGTTCTCC -3'	
SLC26A7	Forward	5'- GAAAATGCCAGCAATCAGCCA -3'	XM_021089130.1
	Reverse	5'- AGGGCCACAGTTCCCATTG -3'	
TKTL2	Forward	5'- CTGGCCTTTGCATCCCACTA -3'	XM_013978706.2
	Reverse	5'- GTATCCATGCAGTGCGCAAG -3'	
ACBD7	Forward	5'- GGAAGATGCCATGAGTGCCT -3'	XM_003357745.4
	Reverse	5'- CTGAGGGCTTCAAAAGGCAAA -3'	
THRSP	Forward	5'- GTAGCCTCGGACTCTAGGCA -3'	NM_001244376.1
	Reverse	5'- CTGCAGGTCCAGGTCTTTCT -3'	
SLPI	Forward	5'- CAAGTGCACAAGTGACTGGC -3'	NM_213870.1
	Reverse	5'- GGCCATAGACCACTGGACAC -3'	
FADS1	Forward	5'- GTCACTGCCTGGCTCATTCT -3'	NM_001113041.1
	Reverse	5'- AGGTGGTTCCACGTAGAGGT -3'	
ACSL6	Forward	5'- GAATACGGGCACTCTCTGGC -3'	XM_021084743.1
	Reverse	5'- CCTAGGACCCCAGTTTGCAG -3'	
FOS	Forward	5'- GACTGCTATCTCGACCAGCC -3'	NM_001123113.1
	Reverse	5'- CTGGCATGGTCTTCACGACT -3'	

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Table 4.

Fatty acid (%)	CP		BP		<i>P</i> -Value
	Mean	sd	Mean	sd	
C10:0	0.08	0.01	0.10	0.01	**
C12:0	0.06	0.01	0.07	0.01	NS
C14:0	1.14	0.17	1.20	0.08	NS
C16:0	23.76	0.66	24.21	0.73	NS
C16:1	3.06	0.46	3.32	2.41	NS
C17:0	0.23	0.03	0.24	0.02	NS
C18:0	11.74	0.79	11.95	0.73	NS
C18:1	41.81	3.20	46.21	1.05	**
C18:2n-6	12.67	2.74	8.74	1.40	**
C18:3n-3	0.27	0.02	0.23	0.04	*
C20:0	0.13	0.01	0.19	0.02	***
C20:1	0.51	0.10	0.70	0.07	***
C20:2	0.29	0.04	0.23	0.02	**
C20:3n-6	0.35	0.11	0.22	0.05	**
C20:4n-6	2.82	0.87	1.53	0.48	**
C22:5	0.28	0.09	0.14	0.04	**
SFA	37.07	1.09	37.96	1.40	NS
UFA	62.04	1.05	61.25	1.34	NS
MUFA	45.32	3.61	50.18	1.21	***
PUFA	3.75	1.06	2.11	0.55	***
n6:n3	27.59	3.63	28.35	3.08	NS

Reference

1. Dransfield E, Ngapo T, Nielsen NA, Bredahl L, Sjöden P-O, Magnusson M, Campo M, Nute G: **Consumer choice and suggested price for pork as influenced by its appearance, taste and information concerning country of origin and organic pig production.** *Meat Science* 2005, **69**(1):61-70.
2. Rosenvold K, Andersen HJMs: **Factors of significance for pork quality—a review.** 2003, **64**(3):219-237.
3. Bonneau M, Lebret BJMs: **Production systems and influence on eating quality of pork.** 2010, **84**(2):293-300.
4. Herrera-Mendez CH, Becila S, Boudjellal A, Ouali AJTiFS, Technology: **Meat ageing: Reconsideration of the current concept.** 2006, **17**(8):394-405.
5. Ekarius C: **Storey's illustrated breed guide to sheep, goats, cattle, and pigs:** Storey Pub.; 2008.
6. Dohner JV: **The encyclopedia of historic and endangered livestock and poultry breeds:** Yale University Press; 2001.
7. Freeman TC, Ivens A, Baillie JK, Beraldi D, Barnett MW, Dorward D, Downing A, Fairbairn L, Kapetanovic R, Raza S: **A gene expression atlas of the domestic pig.** *BMC biology* 2012, **10**(1):90.
8. Bonnet A, Bevilacqua C, Benne F, Bodin L, Cotinot C, Liaubet L, Sancristobal M, Sarry J, Terenina E, Martin P: **Transcriptome profiling of sheep granulosa cells and oocytes during early follicular development obtained by laser capture microdissection.** *BMC genomics* 2011, **12**(1):417.
9. Martin JA, Wang Z: **Next-generation transcriptome assembly.** *Nature Reviews Genetics* 2011, **12**(10):671.
10. Ozsolak F, Milos PM: **RNA sequencing: advances, challenges and opportunities.** *Nature reviews genetics* 2011, **12**(2):87.
11. Sukhija PS, Palmquist D: **Rapid method for determination of total fatty acid content and composition of feedstuffs and feces.** *Journal of Agricultural and Food Chemistry* 1988, **36**(6):1202-1206.
12. Christensen LB: **Drip loss sampling in porcine m. longissimus dorsi.** *Meat science* 2003, **63**(4):469-477.
13. O'fallon J, Busboom J, Nelson M, Gaskins C: **A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs.** *Journal of animal science* 2007, **85**(6):1511-1521.
14. Huang Y, Yan X, Zhao JX, Zhu MJ, McCormick RJ, Ford SP, Nathanielsz PW, Ren J, Du M: **Maternal obesity induces fibrosis in fetal myocardium of sheep.** *American Journal of Physiology - Endocrinology and Metabolism* 2010, **299**(6):E968-E975.
15. Sherman BT, Lempicki RAJNp: **Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.** 2009, **4**(1):44-57.
16. Dennis G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RAJGb: **DAVID: database for annotation, visualization, and integrated discovery.** 2003, **4**(9):R60.
17. Zou T, Wang B, Yang Q, de Avila JM, Zhu MJ, You J, Chen D, Du M: **Raspberry promotes brown and beige adipocyte development in mice fed high-fat diet through activation of AMP-activated protein kinase (AMPK) alpha1.** *The Journal of nutritional biochemistry* 2018,

55:157-164.

18. Bessa R, Hughes R, Jeronimo E, Moreira O, Prates J, Doran OJJoas: **Effect of pig breed and dietary protein level on selected fatty acids and stearyl-coenzyme A desaturase protein expression in longissimus muscle and subcutaneous fat.** 2013, **91**(9):4540-4546.
19. Eagerman B, Clydesdale F, Francis F: **Determination of fresh meat color by objective methods.** *Journal of Food Science* 1977, **42**(3):707-710.
20. Watanabe G, Motoyama M, Nakajima I, Sasaki KJA-Ajoas: **Relationship between water-holding capacity and intramuscular fat content in Japanese commercial pork loin.** 2018, **31**(6):914.
21. Lo L, McLaren D, McKeith F, Fernando R, Novakofski J: **Genetic analyses of growth, real-time ultrasound, carcass, and pork quality traits in Duroc and Landrace pigs: I. Breed effects.** *Journal of animal science* 1992, **70**(8):2373-2386.
22. De Vries A, Van der Wal P, Long T, Eikelenboom G, Merks J: **Genetic parameters of pork quality and production traits in Yorkshire populations.** *Livestock Production Science* 1994, **40**(3):277-289.
23. Bressan MC, Almeida J, Santos Silva J, Bettencourt C, Francisco A, Gama LT: **Carcass characteristics and fat depots in Iberian and F Large White x Landrace pigs intensively finished or raised outdoors in oak-tree forests.** *Journal of animal science* 2016, **94**(6):2592-2602.
24. Tang J, Faustman C, Hoagland TA, Mancini RA, Seyfert M, Hunt MCJJoA, Chemistry F: **Postmortem oxygen consumption by mitochondria and its effects on myoglobin form and stability.** 2005, **53**(4):1223-1230.
25. Ramanathan R, Mancini RAJM, Biology M: **Role of Mitochondria in Beef Color: A Review.** 2018, **2**(1):309-320.
26. Hunt M, Hedrick HJJoFS: **Profile of fiber types and related properties of five bovine muscles.** 1977, **42**(2):513-517.
27. Cho IC, Park HB, Ahn JS, Han SH, Lee JB, Lim HT, Yoo CK, Jung EJ, Kim DH, Sun WS *et al*: **A functional regulatory variant of MYH3 influences muscle fiber-type composition and intramuscular fat content in pigs.** *PLoS genetics* 2019, **15**(10):e1008279.
28. Livingston DJ, Brown WD: **The chemistry of myoglobin and its reactions.** In.; 1981.
29. Wittenberg JB, Wittenberg BAJJoeb: **Myoglobin function reassessed.** 2003, **206**(12):2011-2020.
30. Kim G-D, Jeong J-Y, Hur S-J, Yang H-S, Jeon J-T, Joo S-TJFSOAR: **The relationship between meat color (CIE L* and a*), myoglobin content, and their influence on muscle fiber characteristics and pork quality.** 2010, **30**(4):626-633.
31. Bosch L, Tor M, Reixach J, Estany JJMS: **Age-related changes in intramuscular and subcutaneous fat content and fatty acid composition in growing pigs using longitudinal data.** 2012, **91**(3):358-363.
32. Olivares A, Daza A, Rey A, Lopez-Bote CJMS: **Interactions between genotype, dietary fat saturation and vitamin A concentration on intramuscular fat content and fatty acid composition in pigs.** 2009, **82**(1):6-12.
33. Straadt IK, Aaslyng MD, Bertram HCJMS: **Sensory and consumer evaluation of pork loins from crossbreeds between Danish Landrace, Yorkshire, Duroc, Iberian and Mangalitza.** 2013, **95**(1):27-35.

34. Kris-Etherton PM: **AHA Science Advisory. Monounsaturated fatty acids and risk of cardiovascular disease.** American Heart Association. Nutrition Committee. *Circulation* 1999, **100**(11):1253-1258.
35. Zhao X, Mo D, Li A, Gong W, Xiao S, Zhang Y, Qin L, Niu Y, Guo Y, Liu XJ: **Comparative analyses by sequencing of transcriptomes during skeletal muscle development between pig breeds differing in muscle growth rate and fatness.** 2011, **6**(5).
36. Wang Y, Reverter A, Mannen H, Taniguchi M, Harper G, Oyama K, Byrne K, Oka A, Tsuji S, Lehnert S: **Transcriptional profiling of muscle tissue in growing Japanese Black cattle to identify genes involved with the development of intramuscular fat.** *Australian Journal of Experimental Agriculture* 2005, **45**(8):809-820.
37. Si-Tahar M, Merlin D, Sitaraman S, Madara JL: **Constitutive and regulated secretion of secretory leukocyte proteinase inhibitor by human intestinal epithelial cells.** *Gastroenterology* 2000, **118**(6):1061-1071.
38. Irvin MR, Shrestha S, Chen Y-DI, Wiener HW, Haritunians T, Vaughan LK, Tiwari HK, Taylor KD, Scherzer R, Saag MS: **Genes linked to energy metabolism and immunoregulatory mechanisms are associated with subcutaneous adipose tissue distribution in HIV-infected men.** *Pharmacogenetics and genomics* 2011, **21**(12):798.
39. Fu Y, Li C, Tang Q, Tian S, Jin L, Chen J, Li M, Li C: **Genomic analysis reveals selection in Chinese native black pig.** *Scientific Reports* 2016, **6**:36354.
40. Neess D, Bek S, Engelsby H, Gallego SF, Faergeman NJ: **Long-chain acyl-CoA esters in metabolism and signaling: role of acyl-CoA binding proteins.** *Progress in lipid research* 2015, **59**:1-25.
41. Pena R, Noguera J, Casellas J, D í az I, Fern á ndez A, Folch J, Ib á ñez - Escriche N: **Transcriptional analysis of intramuscular fatty acid composition in the longissimus thoracis muscle of Iberian × Landrace back - crossed pigs.** *Animal genetics* 2013, **44**(6):648-660.
42. Li LO, Klett EL, Coleman RA: **Acyl-CoA synthesis, lipid metabolism and lipotoxicity.** *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids* 2010, **1801**(3):246-251.
43. Reiner G, Heinrich L, Müller E, Geldermann H, Dzapo V: **Indications of associations of the porcine FOS proto - oncogene with skeletal muscle fibre traits.** *Animal genetics* 2002, **33**(1):49-55.

Figures

Figure 1

Effect of breeds on the oxygen consumption (longissimus dorsi) (CP: Cross-bred commercial pigs; BP: British Large Black pigs; NS: not significant/(*), (**), (***) Significant $P < .05, 0.01, 0.001$, respectively)

Figure 2

Effect of breeds on the myoglobin calculation (longissimus dorsi) (CP: Cross-bred commercial pigs, BP: British Large Black pigs; MMb: Metmyoglobin, DMb: Deoxymyoglobin, OMb: Oxymyoglobin; NS: not significant/(*), (**), (***) Significant $P < .05$, 0.01, 0.001, respectively)

Figure 3

Volcano figure of RNA-seq (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs; Up: up regulated in BP compared with CP; Normal: No significant differences; Down: down regulated in BP compared with CP)

Figure 4

Gene ontology (GO) analysis of differentially expressed genes highly express in BP (A) and CP (B). The identified differentially expressed genes were classified in to Biological Process. The numbers of genes the GO term is shown above (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs)

Figure 5

Gene ontology (GO) analysis of differentially expressed genes highly express in BP (A) and CP (B). The identified differentially expressed genes were classified in to Cellular Component. The numbers of genes the GO term is shown above (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs)

A Enriched Go Terms (Molecular Function) in BP

B Enriched Go Terms (Molecular Function) in CP

Figure 6

Gene ontology (GO) analysis of differentially expressed genes highly express in BP (A) and CP (B). The identified differentially expressed genes were classified in to Molecular Function. The numbers of genes the GO term is shown above (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs)

Figure 7

Effect of breeds on the related gene expression (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs) (*) Significant $P < .05$.