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Evaluation of Muscle Chemical Composition, Amino Acids Profile and Antioxidative Capacity of the Shaziling Pig and its Crossbreeds

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ABSTRACT

Background: Meat quality in pigs is an extremely important economic trait. The Shaziling pig is representative of good meat quality but has been scarcely utilized because of the unpleasant growth rate and lean percentage.

Methods: The differences of muscle chemical composition, amino acids profile and antioxidative capacity were evaluated among [(Berkshire × Shaziling) × (Berkshire × Shaziling) (BS × (B × S)], (Berkshire × Shaziling) × Shaziling [(B × S) × S], Shaziling × (Berkshire × Shaziling) [S × (B × S)] and Shaziling (S × S) pigs.

Result: Four groups had plentiful contents of mineral elements (Ca, Zn, Na, K, Cu, Mg, Mn) and abundant amino acids content and no obvious differences in diameters of myofibers and adipocytes were found among four groups. In addition, (B × S) × S pigs had the highest crude protein content, possessed comprehensive nutrient in amino acids and showed preferable antioxidative capacity, suggesting that the meat of (B × S) × S pigs has optimal shelf life.

Key words: Amino acids profile, Antioxidative capacity, Crossbreeds, Muscle chemical composition, Shaziling pigs.

INTRODUCTION

Meat quality in pigs is an extremely important economic trait for breeders, food developers and consumers. With the development of society and the promotion of people's life quality, the focal point of meat consumption demand has gradually altered from 'quantity' to 'quality' (Ilavarasan and Abraham 2018). The Shaziling pig, an indigenous Chinese breed mainly reared in Hunan province, is representative of good meat quality and strong adaptability (Yang *et al*., 2016; Chen *et al*., 2020). However, the Shaziling pig has been scarcely utilized on commercial farms because of the unpleasant traits of growth rate and lean percentage. Nowadays, there is a growing trend to improve the efficiency of commercial pig production by cultivating new breeds with the advantages of indigenous and lean-type pig breeds through crossbreeding systems (Jiang *et al*., 2011; Gopinathan *et al*., 2011; Guo *et al*., 2017).

Our previous study cultivated the crossbreeds of [Berkshire × Shaziling) × (Berkshire × Shaziling) (BS × (B × S)], (Berkshire × Shaziling) × Shaziling [(B × S) × S], Shaziling × (Berkshire × Shaziling) [S × (B × S)] and the pure Shaziling pigs (S × S), respectively. The object of the present research was to evaluate the differences of muscle chemical composition, amino acids profile and antioxidative capacity among four groups were evaluated. This study provides the scientific basis for exploitation of pork products.

MATERIALS AND METHODS

The experiment was performed in accordance with the guidelines for the Animal Care and Use Committee of Hunan Institute of Animal and Veterinary Science in 2020.

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Animals and experimental design

A total of 80 healthy pigs (50% castrated male and 50% female) with similar initial body weight were randomly selected including BS × (B × S), (B × S) × S, S × S and S × (B × S). Each group consisted of two pens with ten pigs each and the pigs were raised under similar conditions. All pigs were hand-fed two times daily and water was provided *ad libitum*. The feeding experiment lasted for 98 days after 7 days of adaptation period. At the end of the experiment, pigs with medium weight per pen were selected (50% castrated male and 50% female) and the slaughter weight was recorded. Pigs were slaughtered at a local commercial slaughter house and subjected to electrical stunning, exsanguination, dehairing, then the carcass was longitudinally and symmetrically split. *Longissimus thoracis* (LT) and *longissimus lumborum* (LL) muscles on the left side of carcass were immediately harvested within 1 hour of postmortem. The ingredient and chemical composition of

the diet offered to all pigs and the information about the experimental pigs are presented in Table 1 and 2 respectively.

Muscle chemical composition

Analysis of water in LL sample was performed in duplicate following the Association of Analytical Chemists methods (Latimer, 2012). Crude protein content was measured using Kjeldahl method (Machado *et al*., 2020). The method of suprapur nitric acid-hydrochloric acid digestion was utilized to detect the contents of mineral elements by ICP-OES on the basis of the Determination of Multiple Elements in Food (GB 5009.268-2016).

Determination of amino acid profile

The composition of amino acids (AAs) in LL sample was detected according to the method previously described (Liu *et al*., 2016) and the content of individual AA was represented with percentage of total amino acids.

Table 1: Ingredient and chemical composition of experimental diet.

Item	Growing (30 kg-60 kg)	Fattening (60 kg-)
Ingredient, %		
Paddy	50.40	50.50
Corn	5.30	7.90
Soybean meal	11.90	5.00
Rice	-	10.00
Wheat bran	6.00	4.60
Oil bran	20.00	18.00
Soybean oil	2.40	-
Premix†	4.00	4.00
Total	100.00	100.00
Chemical composition		
Digestible energy, MJ kg^{-1}	13.02	12.71
Crude protein, %	13.51	11.00
Calcium, %	0.60	0.62
Total phosphorus, %	0.64	0.76
Available phosphorus, %	0.18	0.17
Lysine, %	0.75	0.60
Methionine, %	0.22	0.18

Supplied, per kilogram of diet: 19.8 mg $CuSO_4 \cdot 5H_2O$; 0.20 mg KI; 400 mg $FeSO_4 \cdot 7H_2O$; 0.56 mg $NaSeO_3$; 359 mg $ZnSO_4 \cdot 7H_2O$; 10.2 mg $MnSO_4 \cdot H_2O$; 5 mg vitamin K (menadione); 2 mg vitamin B_1; 15 mg vitamin B_2; 30 µg vitamin B_{12}; 5400 IU vitamin A; 110 IU vitamin D_3; 18 IU vitamin E; 80 mg choline chloride; 100 mg Fungicide.

Histological analysis of muscle and adipose tissues

The diameter of myofibers in LT samples was determined by classic hematoxylin-eosin staining. Briefly, the samples were excised perpendicularly to the direction of the myofibers and serial tissue sections of 10 µm were cut using cryostat. The backfat samples with size of 10 mm × 10 mm × 10 mm were snap-frozen in liquid nitrogen for subsequent Oil Red O staining. The frozen sections were washed with isopropanol and then incubated with Oil Red O solution, then counterstained with hematoxylin and mounted in glycerin jelly. All the above sections were viewed at 100 × magnification using microscope and five areas were randomly selected in each sample for measuring the diameter of myofibers and adipocytes, respectively.

Analysis of antioxidative parameters

The antioxidative parameters were detected in LT and serum samples. Total superoxide dismutase (T-SOD) activity, catalase (CAT) activity and total antioxidative capacity (TAC) were analyzed by automatic biochemical analyzer as per the method of El-Baz *et al*., (2020). The activity of glutathione peroxidase (GSH-Px) and content of malondialdehyde (MDA) were assayed using ELISA method with automatic enzyme-mark analyzer as per the method of Qin *et al*., (2018). These experiments were executed by the corresponding kits following the manufacturer's protocols.

Statistical analysis

Experimental data were analyzed by one-way ANOVA procedure with SPSS 20.0 software. Duncan method was used for multiple comparison and significance test. The result was expressed as mean value and SEM and the difference was considered to be significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Muscle chemical composition

Compared with BS × (B × S) and S × (B × S) pigs, (B × S) × S and Shaziling pigs had higher ($P<0.05$) crude protein content (Table 3). With regard to mineral elements, the content of Sodium in Shaziling pigs was remarkably higher ($P<0.01$) than that in (B × S) × S and S × (B × S) pigs and BS × (B × S) pigs was higher ($P<0.01$) than S × (B × S) pigs. And the content of Potassium in S × (B × S) pigs were lower

Table 2: Informations about experimental pigs.

Item	BS × (B × S)	(B × S) × S	S × S	S × (B × S)	SEM	p-value
Initial body weight, kg	32.40	30.19	31.02	32.27	0.35	0.08
Number	20	20	20	20		
Slaughter weight, kg	92.23[a]	88.30[a]	83.43[b]	90.85[a]	1.03	< 0.01
Number	6	4	4	4		

BS × (B × S), (Berkshire × Shaziling) × (Berkshire × Shaziling); (B × S) × S, (Berkshire × Shaziling) × Shaziling; S × S, Shaziling × Shaziling; S × (B × S), Shaziling × (Berkshire × Shaziling).

[a-e] Within a row, values with different superscript letters differ ($P<0.05$).

(P<0.05) than that in other groups (Table 2). Water is a major component of meat, accounting for approximately 75% of the meat weight (Aboagye *et al*., 2020), the result of this study is in line with this rule, with water content of 72.38%-73.50%. Our study discovered that the content of mineral elements was plentiful and similar to the levels presented by earlier researches (Marchello *et al*., 1985; Lombardi-Boccia *et al*., 2005; Jukna *et al*., 2013; Chen *et al*., 2020), including Large White, Berkshire, Shaziling pigs and their crossbreeds.

Amino acids profile

Only considerable (P<0.05) difference in Histidine content was discovered in all detected AAs (Table 4) and Histidine content in (B × S) × S pigs were higher (P<0.05) than that in BS × (B × S) and S × (B × S) pigs. AAs generally take part in taste and flavors, being precursors of many odorants (Wu *et al*., 2018). Our research verified that aspartic acid, glutamic acid, leucine and lysine were the predominant AAs in each group, among which glutamic acid content was the

Table 3: Muscle chemical composition of BS × (B × S), (B × S) × S, S × S and S × (B × S) pigs.

Item	BS × (B × S)	(B × S) × S	S × S	S × (B × S)	SEM	p-value
Water, %	73.50	72.85	72.63	72.38	0.24	0.34
Crude Protein, %	20.47^{b}	21.15^{a}	21.15^{a}	20.53^{b}	0.11	0.02
Ash, %	1.05	1.03	0.99	1.03	0.01	0.49
Ca, mg/kg	31.95	32.28	29.53	28.68	0.90	0.45
Zn, mg/kg	16.02	15.65	15.55	14.70	0.40	0.72
Na, mg/kg	376.00^{ab}	343.50^{bc}	387.50^{a}	310.00^{c}	9.04	< 0.01
K, %	0.43a	0.42a	0.43a	0.40b	0.00	0.02
Cu, mg/kg	0.43	0.42	0.49	0.49	0.02	0.57
Mg, mg/kg	334.00	332.75	338.25	341.50	9.31	0.99
Mn, mg/kg	0.06	0.06	0.08	0.08	0.00	0.11

BS × (B × S), (Berkshire × Shaziling) × (Berkshire × Shaziling); (B × S) × S, (Berkshire × Shaziling) × Shaziling; S × S, Shaziling × Shaziling; S × (B × S), Shaziling × (Berkshire × Shaziling).

$^{a\text{-}c}$Within a row, values with different superscript letters differ (P < 0.05).

Table 4: Amino acid profile of BS × (B × S), (B × S) × S, S × S and S × (B × S) pigs.

Item	BS × (B × S)	(B × S) × S	S × S	S × (B × S)	SEM	p-value
Aspartic acid, %	1.91	1.98	1.90	1.85	0.02	0.12
Threonine, %	0.92	0.95	0.93	0.90	0.01	0.17
Serine, %	0.67	0.69	0.68	0.66	0.01	0.51
Glutamic acid, %	2.96	3.06	2.95	2.86	0.30	0.18
Glycine, %	0.89	0.91	0.89	0.85	0.01	0.17
Alanine, %	1.17	1.22	1.19	1.1	0.01	0.21
Valine, %	1.11	1.16	1.13	1.09	0.01	0.21
Methionine, %	0.53	0.57	0.53	0.52	0.01	0.36
Isoleucine, %	1.05	1.11	1.08	1.05	0.01	0.21
Leucine, %	1.74	1.82	1.77	1.72	0.02	0.14
Tyrosine, %	0.74	0.78	0.75	0.73	0.01	0.12
Phenylalanine, %	0.89	0.95	0.92	0.90	0.01	0.09
Lysine, %	1.94	2.05	1.99	1.93	0.02	0.08
Histidine, %	1.00^{b}	1.07^{a}	1.02^{ab}	0.98^{b}	0.01	0.03
Arginine, %	1.35	1.39	1.35	1.32	0.01	0.25
Proline, %	0.79	0.83	0.80	0.76	0.01	0.09
Total EAAs†, %	9.18	9.66	9.36	9.08	0.09	0.09
Total FAAs‡, %	14.90	15.50	15.00	14.54	0.14	0.15
Total AAs, %	19.65	20.51	19.86	19.25	0.18	0.12

BS × (B × S), (Berkshire × Shaziling) × (Berkshire × Shaziling); (B × S) × S, (Berkshire × Shaziling) × Shaziling; S × S, Shaziling × Shaziling; S × (B × S), Shaziling × (Berkshire × Shaziling).

$^{a,\ b}$Within a row, values with different superscript letters differ (P < 0.05).

†Total EAAs = Threonine + Valine + Methionine + Isoleucine + Leucine + Phenylalanine + Lysine + Histidine.

‡Total FAAs = Aspartic acid + Serine + Glutamic acid + Glycine + Alanine + Valine + Methionine + Isoleucine + Leucine + Tyrosine + Arginine + Proline.

highest. This finding was in accordance with former investigations obtained in several Chinese indigenous pig breeds, foreign pig breeds and their hybrid pigs and the typical hybrid pig breed Duroc × (Landrace × Large White) (Lu *et al*., 2008; Zhou *et al*., 2016; Chen *et al*., 2020; Chen *et al*., 2021), demonstrating that there is a certain rule in AAs profile in *longissimus dorsi* muscle of pigs. Apart from histidine, there were no significant differences in AAs among groups, including total essential amino acids (EAAs) content, total flavor amino acids (FAAs) content and total AAs content. We also found that almost all AAs were higher in (B × S) × S pigs, but lower in S × (B × S) pigs, albeit not all the differences were to the level of statistical significance, hinting that (B × S) × S pigs possessed comprehensive nutrient in AAs.

Histological analysis of muscle and adipose tissues

As exhibited in Fig 1, there were no obvious differences in diameters of myofibers and adipocytes among four groups. It is certain that the indigenous breed Shaziling pigs are capable of depositing more fat than the lean-type Berkshire pigs and the diameter of adipocytes in backfat tissue was expectedly the highest in Shaziling pigs than that in other three groups, which is in agreement with the results acquired in other indigenous and lean-type pig breeds, such as Chinese Taihu and Landrace, Chinese Meishan and Landrace (Li *et al*., 2008; Nakajima *et al*., 2011). Meanwhile, it is noteworthy that the differences in diameter of adipocytes among four groups were not to the level of statistical significance, probably because of the Shaziling pigs consanguinity contained in three crossbreeds.

Analysis of antioxidative parameters

The activities of antioxidative enzymes in muscle and serum were estimated (Fig 2). GSH-Px, T-SOD and CAT, were the highest in (B × S) × S pigs, but the lowest in BS × (B × S) pigs, albeit not all the differences were to the level of statistical significance. Moreover, TAC in serum was the lowest in BS × (B × S) pigs and MDA content in both muscle and serum were, as expected, the lowest in (B × S) × S pigs, indicating an advanced antioxidative capacity in (B × S) × S pigs. Furthermore, it has been showed that histidine indirectly participates in the antioxidative capacity of meat (Xu *et al*., 2017; Moro *et al*., 2020) and the present research revealed that histidine content in (B × S) × S pigs was the highest. Accordingly, it was speculated that histidine content maybe another potential factor resulting in the difference in antioxidative capacity between four groups. Furthermore, our study hinted that the meat of (B × S) × S pigs is more unsusceptible to oxidative deterioration, which is highly beneficial to the preservation of meat products (Fan *et al*., 2020).

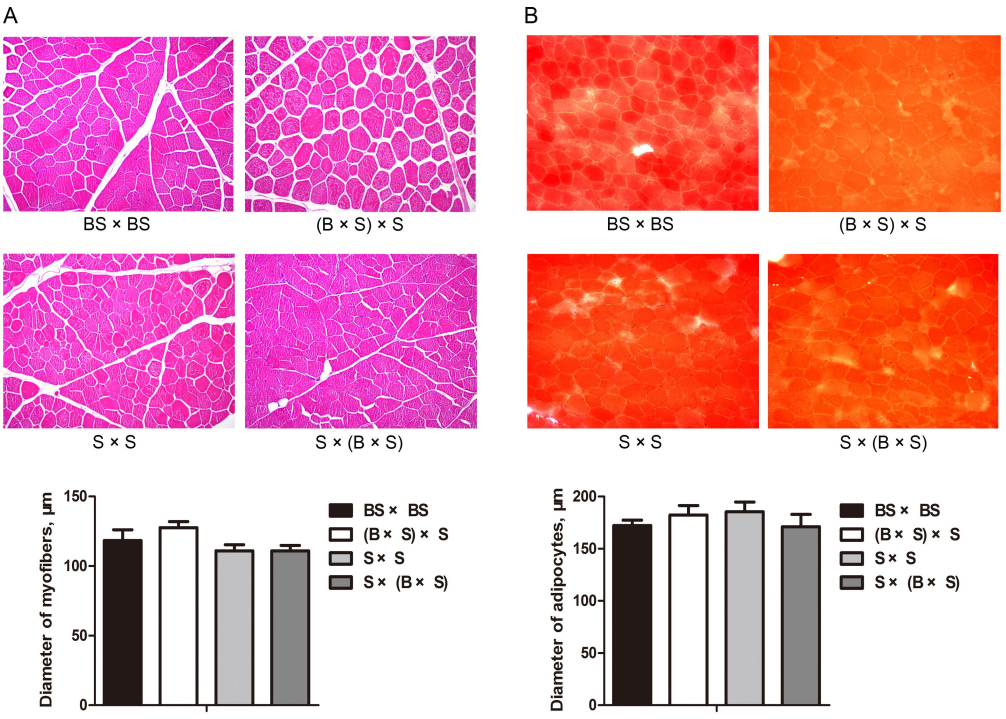

Fig 1: Histological analysis of LT muscle and backfat tissues. A. hematoxylin-eosin micrographs of myofibers (upper) and quantitative analysis of myofibers diameter (bottom) in different groups. B. Cryosectional images of adipocytes (upper) and quantitative analysis of adipocytes diameter (bottom) in different groups.

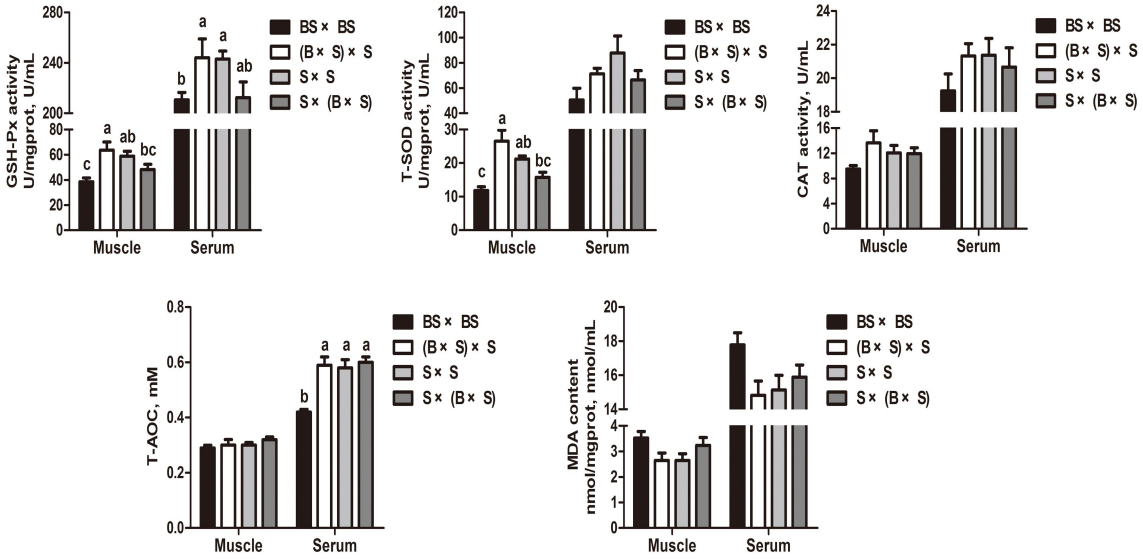

Fig 2: Analysis of antioxidative parameters in LT muscle and serum.

CONCLUSION

Four groups had plentiful content of mineral elements and abundant amino acids. (B × S) × S pigs had highest crude protein content, possessed comprehensive nutrient in amino acids and showed preferable antioxidative capacity, suggesting that the meat of (B × S) × S pigs has optimal shelf life.

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Conflict of interest

The authors declare that there is no conflict of interest.

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