Comparative analysis of amino acid content and protein synthesis-related genes expression levels in breast muscle among different duck breeds/strains

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ABSTRACT Evidences have found important effects of breeds/strains on the content of amino acids (**AAs**) which is an important substrate for protein synthesis and contributes greatly to meat quality. Therefore, the objective of the present study was to compare the AAs content and protein synthesis-related genes expression levels in breast muscle of native breed (Jianchang duck (**J**)), hybrid strains (BH1, BH2, and MC♂ × (BGF2♂ × GF2♀)♀ (**MC**)), and commercial breed (Cherry Verry duck). Results showed that a total of 17 AAs (**TAAs**) was detected from breast muscle among 5 duck breeds/strains including 11 essential AAs (**EAAs**). Among these AAs, the contents of Proline, Threonine, Glutamine, Serine, Methionine, Phenylalanine, Histidine, and Cysteine were significant difference among 5 duck breeds/strains. The contents of EAAs, TAAs, and flavor AAs were higher in breast muscle of J and BH2 than those in other duck breeds/strains, and the ratio of EAAs/TAAs was higher in breast muscle of BH2. Furthermore, the expression levels of eukaryotic translation initiation factor 4E-binding protein 1, mammalian target of rapamycin, and proton-coupled amino acid transporter 1 were the highest in breast muscle of BH2, and that of solute carrier family 38 member 2 was the highest in breast muscle of J. Meanwhile, principal component analysis results showed that principal component 1 of BH1, principal component 3 of BH2, and principal component 2 of MC were positively corelated with EAAs/TAAs, and principal component 1 was positively correlated with flavor AAs and EAAs. In conclusion, compared to BH1, MC, and Cherry Verry duck, AA content was higher in breast muscle of BH2 and J, which might be associated with the higher expression levels of mammalian target of rapamycin, eukaryotic translation initiation factor 4E-binding protein 1, and proton-coupled amino acid transporter 1 in breast muscle of BH2 and solute carrier family 38 member 2 in breast muscle of J. The comparative analysis of AA content in breast muscle among different duck breeds/strains could provide an important basis for improving the nutritional value of duck meat in the breeding process.

Key words: breast muscle, breeds/strains, amino acid, protein synthesis-related genes, duck

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INTRODUCTION

Amino acid is an important composition of proteins, which have important influence on the quality of meat (Dalle Zotte et al., 2020; Haraf et al., 2021). Increasing evidence has shown that amino acid had different metabolic functions and nutritional value in meat (Young and Pellett, 1984; Wu, 2009). For example, Glutamate (**Glu**) regulated cells glycolysis and assisted in ammonia detoxification (Brosnan, 2001), Phenylalanine (**Phe**) was a precursor for the synthesis of thyroid hormones (Suenaga et al., 2008), and Metabolite (**Met**) contributed to the function of anti-oxidative and anti-inflammatory reactions. Glycine was the breakdown product of Threonine (**Thr**) and Serine (**Ser**) (Hilliar et al., 2019), which could increase nitrogen utilization (Siegert and Rodehutscord, 2019). Furthermore, the nutritional value of meat was determined by the quantity of the essential amino acids (**EAAs**). Subramaniyan *et al.* (2016) found that Berkshire pig had better nutritional value of meat due to the higher content of EAAs, the similar results were also observed in chickens (Tang et al., 2021). Meanwhile, the higher content of flavor amino acids (**FAAs**) also improved the nutritional value and quality of pig meat (Yu et al., 2020). Notably, the

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difference of amino acid content was affected by breeds. Results showed that amino acid content was significant difference in meat of pigs (Chen and Sui, 2018; Xu et al., 2019), rabbits (Nasr et al., 2017; Simonová et al., 2020), chickens (Liu et al., 2021; Yu et al., 2021), and geese (Gumułka and Połtowicz, 2020; Sabow, 2020). However, comparative analysis of amino acid content among different breeds/strains duck have yet been performed.

As substrates for protein synthesis, amino acid regulated gene expression in animal cells at the level of transcription, translation and post-translational protein modification (Wu, 2009). The mammalian target of rapamycin (**mTOR**) signaling pathway was considered to be the primary mechanism for regulating protein synthesis (Wu, 2009). In skeletal muscle cells, amino acid influenced the phosphorylation status and function of many proteins involved in mRNA translation though mTOR signaling pathway, including *mTOR*, S6 kinase1 (***S6K1***) and eukaryotic translation initiation factor 4E-binding protein 1 (***4E-BP1***) (Gingras et al., 1999; Avruch et al., 2001; Beugnet et al., 2003; Wu, 2009). In addition, amino acid transporters also played a key role in mTOR signal transduction and muscle protein synthesis/muscle growth, such as solute carrier family 38 member 2 (***SLC38A2***), solute carrier family 7 member 5 (***SLC7A5***), and proton-coupled amino acid transporter 1 (***PAT1***) (Hyde et al., 2005; Evans et al., 2007; Nicklin et al., 2009; Heublein et al., 2010). However, the regulation mechanism of protein synthesis in breast muscle of ducks was still unclear.

Currently, duck has been becoming one of the most popular poultry commodities behind chicken in Asia. Therefore, the objectives of this study were to detect the amino acid content and expression levels of protein synthesis-related genes in breast muscle among 5 different duck breeds/strains. These results would provide the basic evidence for breeding of ducks.

MATERIALS AND METHODS

Ethics Statement

All animal handling procedures were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University (Chengdu campus, Sichuan, China, Permit No. DKY20170913).

Animals and Sample Collection

In the present study, 100 healthy 56-day-old ducks were selected. These ducks consisted of Nonghua ducks (BH1, BH2, and MC♂ × (BGF2♂ × GF2♀)♀ (**MC**)), Cherry Valley duck (**CV**) and Jianchang duck (**J**), which provided by Waterfowl Breeding Experimental Farm of Sichuan Agricultural University (Sichuan, China). Each breed/strain contained 20 ducks including 10 females and 10 males. All ducks were raised in floor with natural light and free water, and fed with the same feed as in Table 1. After 56 days, these ducks were fasted for 12 h and slaughtered to obtain the breast muscle (pectoralis major) from the left side. The half of breast muscle was stored at -20°C for analysis of amino acids, and the other half stored at -80°C for RNA extraction.

Table 1. Ingredients and nutrients composition of basal diets in 5 duck breeds/strains.

Items	Stage (15−56 d)
Ingredients	
Corn (%)	57.70
Soybean meal (%)	27.50
Wheat middling (%)	7.50
Wheat bran (%)	2.00
Calcium hydrogen phosphate (%)	1.62
Soybean oil (%)	1.40
Limestone powder (%)	0.93
NaCl (%)	0.35
Vitamin and mineral premix (%)	1.00
Total (%)	100
Nutrients	
Metabolizable energy (Mcal/kg)	2,900
Dry matter (%)	87.12
Crude protein (%)	17.50
Crude fat (%)	4.13
Crude fiber (%)	3.00
Calcium (%)	0.85
Total phosphorus (%)	0.65
Available phosphorus (%)	0.40
Lysine (%)	0.85
Methionine (%)	0.40
Methionine + cystine (%)	0.70
Threonine (%)	0.60
Tryptophan (%)	0.19

Analysis of Amino Acid Content

Amino acid content of breast muscle was analyzed by LA8080 amino acid analyzer (HITACHI, Tokyo, Japan) to evaluate the biological value of muscle protein. Briefly, approximately 100 mg of breast muscle sample was ground into mud. Then, transfer the meat mud into an acid hydrolysis bottle which contained 15 mL of HCL (6 mol/L) and add phenol dropwise. After filling with nitrogen, the mixture was hydrolyzed at 110°C for 22 h. Subsequently, the hydrolysate was transferred into a 50 mL volumetric flask and diluted with ultrapure water in a calibrate tube. The solution was filtered into an autosampler vial using a 0.22 μm membrane filter. The various amino acids were separated by ion-exchange chromatography using lithium citrate buffer. Derivatives were detected at 570 and 440 nm after post-column derivatization with ninhydrin.

Total RNA Extraction and Detection of Genes Expression Level

Total RNA from breast muscle of each duck (a total of 30 samples) was extracted by using Trizol (Takara, China) following the manufacturer's instructions. The quality of RNA was evaluated by A260/A280 ratio, and its integrity was detected by 1.5% agarose gel. After that, 1 μg of total RNA was reverse transcribed into complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis Kit (Takara, China). Then, the

Table 2. Primer information used for RT-qPCR of 5 duck breeds/strains in this study.

Genes[1]	Forward (5′-3′)	Reverse (5′-3′)	TM (°C)
mTOR	CTATCTGCCTCAGCTCATTCCT	GTCATCCAGGTTAGCTCCAAAG	52.7
S6K1	ATAATCGTGCTGTGGACTGGTG	TCTGGCTTCTTGTGTGAGGTAGG	61.1
PAT1	GCGAGGACTACAACGACTA	GGAACCTGTAGCAGAAGTGA	57.2
SLC7A5	ACAGTGGTCTTTTTGCCTATGG	CGACAGGGTTGTGAAGTAAGC	52.1
4E-BP1	ACCTTCTGACCTTCCCGACATT	CCAACGCTGGGTTTCTCAT	61.0
SLC38A	TCAGATTCCTTGCCCTATGG	GCAGGCATCATCACTTATGTT	54.5
18S	TTGGTGGAGCGATTTGTC	ATCTCGGGTGGCTGAACG	55.0
β-ACTIN	GCTATGTCGCCCTGGATTTC	CACAGGACTCCATACCCAAGAA	61.0

[1]*mTOR*, mammalian target of rapamycin; *S6K*, S6 kinase1; *PAT1*, proton-coupled amino acid transporter 1; *SLC7A5*, solute carrier family 7 member 5; *4E-BP1*, eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1; *SLC38A2*, solute carrier family 38 member 2.

primers were confirmed to be specific for the target gene by BLAST search. The expression levels of genes involved in protein synthesis were measured in breast muscle by the real-time quantitative polymerase chain reaction (**RT-qPCR**). RT-qPCR was carried out in a CFX96TM Real-Time System (Bio-Rad, USA) using SYBR PrimerScriptTM RT-PCR kit (Takara, China). The volume of total reaction was 12.5 μL, including 6.25 μL SYBR Premix ExTaq II, 1 μL of each primers pair and 4.25 μL cDNA template. The PCR procedures were 95°C for 3 min, followed by 40 cycles of 95°C for 5 s, annealing temperature for 30 s and 72°C for 10 min. An 80-cycle melting curve was carried out, starting at a temperature of 65°C and increasing by 0.5°C every 10 s to determine primer specificity. Each sample was in triplicate and the expression levels of genes were calculated by $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001). *18S* and *β-ACTIN* were considered as housekeeping genes. Primers used for RT-qPCR were listed in Table 2.

Statistical Analysis

The comparative analysis of amino acid content and expression levels of genes, amino acid principal component analysis (**PCA**) and correlation analysis were performed by SPSS 24.0 among 5 duck breeds/strains. The normal distribution and homogeneity of variances of data were tested with Shapiro-Wilk test (Shapiro and Wilk, 1965) and Levene's test (Wu et al., 2003). Multiple comparisons were analyzed using ANOVA followed by Duncan test. $P < 0.05$ was considered to be statistically significant. Data was displayed as mean ± SEM.

RESULTS

Amino Acid Content in Breast Muscle of 5 Duck Breeds/Strains

As shown in Table 3, a total of 17 amino acids were detected from the breast muscle among 5 duck breeds/strains including 11 EAAs. Among these total amino acids (**TAAs**), the contents of Proline (**Pro**), Cysteine (**Cys**), Histidine (**His**), Thr, Met, Phe, Glu, and Ser were significantly different among 5 duck breeds/strains.

Among the EAAs, specifically, the contents of Pro in breast muscle of J, BH1, and BH2 were significantly higher than in that of CV ($P < 0.05$), and was not significant difference between MC and CV or J, BH1, BH2. Compared with J, the content of Thr in breast muscle was significantly higher in MC ($P < 0.05$). In addition, compared with BH1, the content of Met in breast muscle was significantly higher than in CV ($P < 0.05$), and there was no significant difference for content of Met between J, BH2, MC and CV or BH1. For Phe, its content in breast muscle of BH1 and BH2 was significantly higher than in that of MC and CV, and was the lowest in CV ($P < 0.05$). Meanwhile, the content of His was in breast muscle of MC and J significantly higher than in that of BH1 and BH2 ($P < 0.05$), and the content of His in CV was not significant difference with other 4 duck breeds/strains.

Regarding the non-essential amino acids, compared with BH1, the content of Glu in breast muscle was significantly higher than in J ($P < 0.05$). The content of Ser in breast muscle of J was the highest ($P < 0.05$), and there was no significant difference among other 4 breeds/strains of duck. The content of Cys in breast muscle of CV, BH1, and BH2 was significantly higher than in that of MC ($P < 0.05$), and was not significant difference between J and other 4 breeds/strains of duck. Furthermore, the ratio of EAAs and TAAs (**EAAs/TAAs**) in breast muscle of BH1 and BH2 was significantly higher than in that of CV and J ($P < 0.05$). Taken together, except for J, the contents of amino acids in breast muscle of BH2 were the highest compared to BH1, CV, and MC.

PCA of Amino Acid Content

Though the significance analysis of the amino acid content of 5 different duck breeds/strains, it was found that there was information overlap among the amino acids. Therefore, the 17 detected amino acid components were simplified into a group of independent but dominant comprehensive indicators through PCA. According to the eigenvalue greater than 1 and the cumulative contribution rate, 2 principal components (**PCs**) of BH1 ([**BPC**]1 and BPC2), MC ([**MPC**]1 and MPC2), CV ([**CPC**]1 and CPC2), and J ([**JPC**]1 and JPC2) were observed in breast muscle, while 3 PCs of BH2 ([**HPC**]1, HPC2, and HPC3) were observed in breast muscle. It could be seen that the cumulative contribution rates of PC in breast muscle of BH1, BH2, MC, CV and J were 91.05%, 89.32%, 81.47%, 90.45% and 90.70%, respectively (Table 4). Meanwhile, the variance of PC1 was mainly caused by the similar

Table 3. Amino acid content (g/100 g) in breast muscle of 5 duck breeds/strains.

Items[1]	Breeds/strains[2]				
	BH1	BH2	MC	CV	J
EAAs					
Arg	1.108 ± 0.019	1.131 ± 0.016	1.140 ± 0.014	1.131 ± 0.021	1.162 ± 0.021
Pro	0.717 ± 0.015[a]	0.730 ± 0.014[a]	0.700 ± 0.014[ab]	0.657 ± 0.022[b]	0.713 ± 0.015[a]
Gly	0.808 ± 0.011	0.817 ± 0.021	0.779 ± 0.014	0.766 ± 0.017	0.814 ± 0.016
Thr	0.865 ± 0.013[ab]	0.870 ± 0.012[ab]	0.833 ± 0.039[b]	0.861 ± 0.014[ab]	0.905 ± 0.015[a]
Val	0.921 ± 0.014	0.935 ± 0.014	0.922 ± 0.011	0.921 ± 0.017	0.925 ± 0.015
Met	0.225 ± 0.025[b]	0.290 ± 0.026[ab]	0.276 ± 0.015[ab]	0.300 ± 0.019[a]	0.234 ± 0.020[ab]
Ile	0.808 ± 0.015	0.825 ± 0.014	0.824 ± 0.011	0.818 ± 0.017	0.814 ± 0.015
Leu	1.546 ± 0.024	1.544 ± 0.024	1.525 ± 0.018	1.510 ± 0.027	1.567 ± 0.026
Phe	0.741 ± 0.012[a]	0.717 ± 0.014[a]	0.678 ± 0.008[bc]	0.668 ± 0.013[c]	0.709 ± 0.012[ab]
Lys	1.632 ± 0.029	1.663 ± 0.025	1.629 ± 0.020	1.627 ± 0.027	1.669 ± 0.027
His	0.469 ± 0.011[b]	0.467 ± 0.007[b]	0.517 ± 0.009[a]	0.497 ± 0.013[ab]	0.509 ± 0.011[a]
NEAAs					
Glu	2.586 ± 0.041[b]	2.658 ± 0.040[ab]	2.650 ± 0.034[ab]	2.650 ± 0.044[ab]	2.727 ± 0.045[a]
Asp	1.707 ± 0.026	1.731 ± 0.024	1.737 ± 0.020	1.715 ± 0.028	1.769 ± 0.028
Ala	1.151 ± 0.015	1.151 ± 0.018	1.131 ± 0.012	1.125 ± 0.019	1.160 ± 0.019
Ser	0.722 ± 0.010[b]	0.712 ± 0.015[b]	0.717 ± 0.008[b]	0.705 ± 0.012[b]	0.759 ± 0.012[a]
Cys	0.166 ± 0.066[a]	0.167 ± 0.006[a]	0.146 ± 0.004[b]	0.168 ± 0.005[a]	0.154 ± 0.007[ab]
Tyr	0.594 ± 0.012	0.593 ± 0.011	0.583 ± 0.009	0.584 ± 0.012	0.592 ± 0.012
TAAs	16.762 ± 1.248	16.998 ± 1.134	16.783 ± 0.970	16.699 ± 1.400	17.177 ± 1.338
FAAs	7.585 ± 0.114	7.666 ± 0.116	7.557 ± 0.092	7.507 ± 0.131	7.769 ± 0.129
EAAs	9.838 ± 0.762	9.987 ± 0.678	9.820 ± 0.604	9.754 ± 0.876	10.018 ± 0.800
EAAs/TAAs	0.587 ± 0.003[a]	0.588 ± 0.004[a]	0.585 ± 0.006[ab]	0.584 ± 0.005[b]	0.583 ± 0.003[b]

[a-c]Different lowercase letters indicated that the results of post hoc analysis were significant ($P < 0.05$).

[1]Asp, Asparagine; Thr, Threonine; Ser, Serine; Glu, Glutamine; Gly, Glycine; Ala, Alanine; Cys, Cysteine; Val, Valine; Met, Methionine; Ile, Isoleucine; Leu, Leucine; Tyr, Tyrosine; Phe, Phenylalanine; Lys, Lysine; His, Histidine; Arg, Arginine; Pro, Proline.

[2]MC, MC♂ × (BGF2♂ × GF2♀)♀; CV, Cherry Valley duck; J, Jianchang duck; EAAs, essential amino acids; NEAAs, non-essential amino acids; TAAs, total amino acids; FAAs, flavor amino acids.

composition of high positive load amino acid such as Aspartic, Ser, Glu, Alanine, Valine, Isoleucine, Leucine, Tyrosine, Phe, Lysine, His, Arginine, and Pro (Table 5). It was worth noting that HPC2, CPC2, and JPC2 were contributed by similar amino acids (Cys and Met).

The Relative Expression Levels of Protein Synthesis-related Genes

The mRNA levels of protein synthesis-related genes were further detected among 5 duck breeds/strains. As shown in Figure 1, the mRNA expression level of *4E-BP1* in breast muscle of BH2 was significantly higher than in that of CV ($P < 0.05$), there were no significant differences between BH2 or CV and other 3 duck breeds/strains. The mRNA expression level of *mTOR* in breast muscle of BH2 was significantly higher than in that of CV and BH1 ($P < 0.05$), and was no significant differences between BH2, CV and MC, J. The mRNA expression level of *PAT1* in breast muscle of BH2 was significantly higher than in that of other 4 duck breeds/strains ($P < 0.05$), and there were no significant differences among other 4 duck breeds/strains. In addition, the mRNA expression level of *SLC38A2* in breast muscle of J was significantly higher than in that of other 4 duck breeds/strains ($P < 0.05$), and the mRNA expression levels in MC and CV was significantly higher than that in BH1. Notably, the mRNA expression levels of *S6K* and *SLC7A5* were no significant differences among 5 duck breeds/strains. Taken together, the mRNA expression levels of *4E-BP1, mTOR, PAT1*, and *S6K* were the highest in breast muscle of BH2, while that of *SLC38A2* and *SLC7A5* were the highest in breast muscle of J and MC, respectively.

The Pearson's Correlation of Amino Acid PC with Amino Acid Content and Protein Synthesis-related Genes Expression levels

Based on the factor score coefficient (Supplementary Table 1), the PC values of 5 different duck breeds/

Table 4. Eigenvalues of the correlation matrix of the 5 duck breeds/strains.

Items[1]	Eigenvalue	Variance contribution (%)	Cumulative contribution (%)
BH1			
BPC1	14.01	82.44	82.44
BPC2	1.47	8.62	91.05
BH2			
HPC1	12.00	70.57	70.57
HPC2	1.98	11.63	82.20
HPC3	1.21	7.12	89.32
MC			
MPC1	12.51	73.57	73.57
MPC2	1.34	7.90	81.47
CV			
CPC1	14.17	83.38	83.38
CPC2	1.20	7.07	90.45
J			
JPC1	14.22	83.64	83.64
JPC2	1.20	7.06	90.70

[1]MC, MC♂ × (BGF2♂ × GF2♀)♀; CV, Cherry Valley duck; J, Jianchang duck; BPC, principal components of BH1; HPC, principal components of BH2; MPC, principal components of MC♂ × (BGF2♂ × GF2♀)♀; CPC, principal components of CV; JPC, principal components of J.

Table 5. Statistical loadings of variables from PCA of the 5 different duck breed/strains.

	BH1[2]		BH2[2]			MC[2]		CV[2]		J[2]	
Items[1]	BPC1	BPC2	HPC1	HPC2	HPC3	MPC1	MPC2	CPC1	CPC2	JPC1	JPC2
Asp	**0.988**	-0.008	**0.983**	-0.053	-0.064	**0.980**	0.040	**0.994**	-0.034	**0.992**	-0.085
Thr	**0.987**	-0.019	**0.977**	-0.029	-0.168	0.277	**0.286**	**0.991**	-0.015	**0.985**	-0.135
Ser	**0.987**	0.004	**0.818**	-0.120	-0.137	**0.927**	0.088	**0.987**	0.032	**0.987**	-0.063
Glu	**0.967**	0.004	**0.963**	-0.087	-0.189	**0.952**	0.090	**0.985**	-0.040	**0.987**	-0.046
Gly	**0.652**	0.597	0.613	-0.163	**0.737**	0.587	**0.641**	**0.795**	-0.381	**0.781**	0.058
Ala	**0.987**	0.105	**0.953**	-0.095	0.227	**0.957**	0.202	**0.987**	-0.128	**0.979**	-0.071
Cys	**0.635**	-0.524	0.079	**0.790**	0.239	**0.548**	-0.328	0.540	**0.547**	0.573	**0.611**
Val	**0.974**	-0.043	**0.979**	-0.001	-0.059	**0.962**	-0.166	**0.964**	-0.129	**0.983**	-0.119
Met	**0.695**	-0.622	0.188	**0.888**	-0.115	**0.684**	-0.520	0.567	**0.633**	0.480	**0.772**
Ile	**0.991**	-0.021	**0.974**	-0.008	-0.162	**0.986**	-0.116	**0.984**	-0.056	**0.978**	-0.073
Leu	**0.992**	-0.004	**0.980**	-0.021	-0.148	**0.989**	-0.027	**0.991**	-0.033	**0.983**	-0.122
Tyr	**0.956**	-0.234	**0.750**	0.634	-0.085	**0.963**	-0.193	**0.820**	0.507	**0.915**	0.331
Phe	**0.945**	0.143	**0.903**	0.077	-0.094	**0.992**	-0.029	**0.977**	-0.068	**0.975**	-0.141
Lys	**0.901**	0.095	**0.941**	-0.171	-0.128	**0.977**	0.002	**0.988**	-0.024	**0.986**	-0.065
His	**0.942**	-0.076	**0.739**	-0.251	-0.095	**0.617**	-0.361	**0.831**	-0.200	**0.863**	-0.090
Arg	**0.983**	0.028	**0.974**	0.028	0.133	**0.987**	0.080	**0.990**	-0.006	**0.982**	0.018
Pro	**0.691**	0.585	**0.726**	0.036	0.589	**0.761**	0.443	**0.943**	-0.118	**0.913**	-0.115

[1]Asp, Asparagine; Thr, Threonine; Ser, Serine; Glu, Glutamine; Gly, Glycine; Ala, Alanine; Cys, Cysteine; Val, Valine; Met, Methionine; Ile, Isoleucine; Leu, Leucine; Tyr, Tyrosine; Phe, Phenylalanine; Lys, Lysine; His, Histidine; Arg, Arginine; Pro, Proline.

[2]MC, MC♂ × (BGF2♂ × GF2♀)♀; CV, Cherry Valley duck; J, Jianchang duck; BPC, principal components of BH1; HPC, principal components of BH2; MPC, principal components of MC♂ × (BGF2♂ × GF2♀)♀; CPC, principal components of CV; JPC, principal components of J.The **loading** displayed in boldface were variables contributed greatly to the principal components.

strains were calculated. Subsequently, the correlation of amino acid PC with amino acid content and protein synthesis-related genes expression levels was shown in Table 6. There was a negative correlation between BPC1 and the mRNA level of *mTOR* ($P < 0.05$). Meanwhile, PC1s were positively correlated with FAAs and EAAs ($P < 0.05$), and BPC1, HPC3, MPC2, and CPC1 were positively correlated with EAAs/TAAs ($P < 0.05$). Moreover, MPC2 was positively correlated with EAAs ($P < 0.05$), and CPC2 was negatively correlated with FAAs ($P < 0.05$).

DISCUSSION

Poultry meat is a valuable food source, which is rich with many essential nutrients including protein (EAAs), minerals, vitamins and fat (Bogosavljevi-Bokovi et al., 2010). Among these essential nutrients, the contents of amino acids were an important factor to influence the nutritional value of meat (Brzostowski and Tański, 2006). A previous study showed that the nutritional value of protein in food was determined by the quantity of the EAAs (Lund et al., 2011). In the present study, the ratio of EAAs/TAAs in breast muscle of BH2 was significantly higher than other breeds/strains. Further analysis showed that there were significantly difference in the contents of Pro, Thr, Met, Phe, His, Glu, Ser and Cys among 5 duck breeds/strains. Previous study showed that Phe, Met, and His imparted a bitter taste, Glu imparted a pleasant fresh taste, and Ser imparted a sweet taste (Lorenzo and Franco, 2012). Thr was considered as the most deficient amino acid in the human diet, and high content of Thr was very beneficial to the nutrition of poultry meat (Leeson, 1993; Gumułka and Połtowicz, 2020). Meanwhile, the breakdown products of Thr and Ser contributed to nitrogen preference, and Glu was also associated with cellular glycolysis (Brosnan, 2001; Hilliar et al., 2019; Siegert and Rodehutscord, 2019). In the present study, the contents of Glu, Ser, and Thr were higher in breast muscle of J and BH2. In addition, the contents of amino acids were the highest in breast muscle of J and BH2 compared with BH1, CV, and MC, which was accordance with previous studies that native and hybrid breeds had higher amino acids contents (Nasr et al., 2017; Ali et al., 2021). These results suggested that the breast muscle of J and BH2 might have higher amino acid content and better nutritional value.

Increasing evidences have shown that several amino acids could stimulate the phosphorylation of mTOR-sensitive complex 1 in a cell-specific manner to regulate intracellular protein turnover (Escobar et al., 2005; Escobar et al., 2006). Moreover, *mTOR* regulated the protein synthesis through phosphorylating its downstream effectors *4E-BP1* and *S6K1* (Bröer and Bröer, 2017). Other results also showed that a mixture of amino acids or individual amino acid could directly promote the phosphorylation of 4E-BP1 in HEK293 and CHO-IR cells (Hara et al., 1998; Wang et al., 1998). Guo *et al.* (2018) showed that Phe regulated the synthesis or secretion of α-amylase, trypsin and lipase through *S6K1* and *4E-BP1* in pancreatic acinar cells. In the present study, the mRNA expression levels of *mTOR, S6K*, and *4E-BP1* were the highest in breast muscle of BH2. These results suggested that amino acids in the breast muscle of BH2 might promote the protein synthesis *via* mTOR/4E-BP1/S6K signaling pathway. Meanwhile, the mRNA expression levels of amino acid transporters were also detected in the present study, which BH2 had the highest mRNA expression level of *PAT1*, and J had the highest levels of *SLC38A2* and *SLC7A5* in breast muscle. *SLC38A2* could transport small amino acid (such as

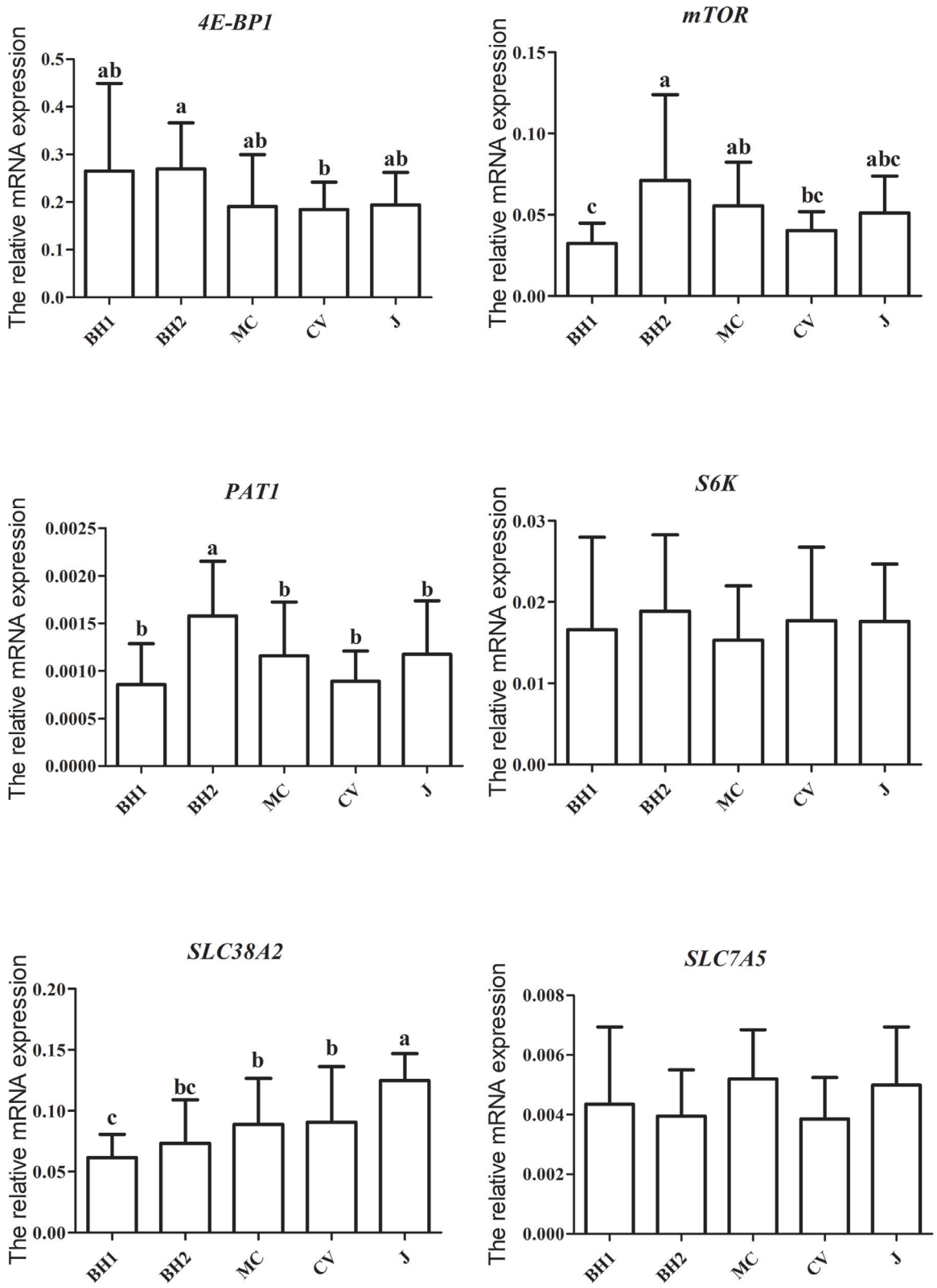

Figure 1. ***The relative expression levels of crucial genes among 5 duck breeds/strains.*** Data was displayed as "Means ± SD" in figures. Abbreviations: *4E-BP1*, eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1; *mTOR*, mammalian target of rapamycin; *PAT1*, proton-coupled amino acid transporter 1; *S6K*, S6 kinase1; *SLC38A2*, solute carrier family 38 member 2; *SLC7A5*, solute carrier family 7 member 5; MC, MC♂ × (BGF2♂ × GF2♀)♀; CV, Cherry Valley duck; J, Jianchang duck. [a-c] Different lowercase letters indicated that the results of post hoc analysis were significant ($P < 0.05$).

Glu) with one sodium ion into muscle fibers, and then exchange them into larger amino acids by *SLC7A5*, which was essential for protein synthesis (Hundal and Taylor, 2009). Decreased *SLC38A2* expression could inhibit the availability of small amino acids, leading to reduced transport by *SLC7A5* of branched chain amino acids (Pearson et al., 2021). *PAT1* was involved in the absorption of small amino acids and their derivatives at

Table 6. The Pearson's correlation analysis of principal composition and genes expression as well as amino acid content.

	BH1[2]		BH2[2]			MC[2]		CV[2]		J[2]	
Items[1]	BPC1	BPC2	HPC1	HPC2	HPC3	MPC1	MPC2	CPC1	CPC2	JPC1	JPC2
Genes											
4E-BP1	0.524	-0.522	-0.004	-0.197	-0.444	0.046	0.527	0.105	0.212	0.544	-0.809
mTOR	-0.847[3]	0.075	0.223	-0.322	-0.337	0.369	-0.483	0.700	-0.230	0.657	-0.452
PAT1	-0.579	0.562	-0.120	-0.783	0.079	-0.327	0.044	0.258	-0.048	0.152	-0.328
S6K	0.194	-0.246	0.413	-0.165	-0.493	0.357	-0.367	-0.263	0.465	0.691	-0.498
SLC38A2	-0.293	0.743	-0.026	0.063	0.531	-0.257	0.539	-0.363	0.118	-0.412	-0.398
SLC7A5	-0.651	0.190	0.406	0.075	-0.471	0.249	0.005	0.761	0.301	0.261	-0.229
Amino acid content											
FAAs	0.996[3]	0.010	0.993[3]	-0.151	-0.089	0.988[3]	0.442	0.996[3]	-0.450[4]	0.997[3]	-0.302
EAAs	0.998[3]	-0.041	0.986[3]	-0.027	-0.113	0.966[3]	0.489[4]	0.997[3]	-0.411	0.997[3]	-0.256
EAAs/TAAs	0.529[4]	0.271	0.057	0.342	0.458[4]	0.219	0.445[4]	0.713[3]	-0.403	0.338	0.088

[1]*4E-BP1*, eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1; *mTOR*, mammalian target of rapamycin; *PAT1*, proton-coupled amino acid transporter 1; *S6K*, S6 kinase1; *SLC38A2*, solute carrier family 38 member 2; *SLC7A5*, solute carrier family 7 member 5; FAAs, flavor amino acids; EAAs, essential amino acid; TAAs, total amino acids.

[2]MC, MC♂ × (BGF2♂ × GF2♀)♀; CV, Cherry Valley duck; J, Jianchang duck; BPC, principal components of BH1; HPC, principal components of BH2; MPC, principal components of MC♂ × (BGF2♂ × GF2♀)♀; CPC, principal components of CV; JPC, principal components of J.

[3]represented the significant difference at $P < 0.01$.

[4]represented the significant difference at $P < 0.05$.

the apical membrane of intestinal epithelial cells (Boll et al., 2004). In the present study, the contents of several amino acids were positive correlation with the expression of *PAT1*, especially in J, which indicated that the high amino acid content of breast meat in J was associated with the absorption of amino acids. Taken together, results from the present study suggested that the differences of amino acids in breast muscle of different duck breeds/strains might be related to the activity of mTOR pathway as well as the transport and absorption of amino acids.

In the present study, correlation analysis showed that BPC1 was negatively correlated with *mTOR*, and HPC1 and JPC1 were positively correlated with *S6K*, which suggested that the amino acid might promote the proteins synthesis through mTOR/4E-BP1/S6K signaling pathway in breast muscle of BH2 and J. Meanwhile, HPC1, CPC1, and JPC1 were positively correlated with *SLC7A5*, indicted that the synthesis of larger amino acids in breast muscle of BH2, CV, and J was promoted, which was conducive to protein synthesis. Notably, BPC1, HPC3 and MPC2 were positively corelated with EAAs/TAAs, which suggested that the ratio of EAAs/TAAs might be improved in breast muscle of hybrid ducks.

In conclusion, compared to BH1, MC, and CV, the contents of EAAs, FAAs, and functional amino acids was higher in breast muscle of BH2 and J, which might be associated with the higher expression levels of *mTOR, 4E-BP1*, and *PAT1* in breast muscle of BH2 and *SLC38A2* in breast muscle of J. Therefore, the breast muscle of BH2 and J had better nutritional value. The comparative analysis of amino acid content in breast muscle among different duck breeds/strains could provide an important basis for improving the nutritional value of duck meat in the breeding process.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

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REFERENCES

Ali, M., K. H. Baek, S. Y. Lee, H. C. Kim, J. Y. Park, C. Jo, J. H. Jung, H. C. Park, and K. C. Nam. 2021. Comparative meat qualities of boston butt muscles (M. subscapularis) from different pig breeds available in Korean market. Food Sci. Anim. Resour. 41:71–84.

Avruch, J., C. Belham, Q. Weng, K. Hara, and K. Yonezawa. 2001. The p70 S6 kinase integrates nutrient and growth signals to control translational capacity. Prog. Mol. Subcell. Biol. 26:115–154.

Beugnet, A., A. R. Tee, P. M. Taylor, and C. G. Proud. 2003. Regulation of targets of mTOR (mammalian target of rapamycin) signalling by intracellular amino acid availability. Biochemical. J. 372:555–566.

Bogosavljevi-Bokovi, S., Z. Pavlovski, M. D. Petrovi, V. Doskovi, and S. Rakonjac. 2010. Broiler meat quality: proteins and lipids of muscle tissue. Afr. J. Biotechnol. 9:9177–9182. http://www.academicjournals.org/AJB.

Boll, M., H. Daniel, and B. Gasnier. 2004. The SLC36 family: proton-coupled transporters for the absorption of selected amino acids from extracellular and intracellular proteolysis. Pflugers. Arch. 447:776–779.

Bröer, S., and A. Bröer. 2017. Amino acid homeostasis and signalling in mammalian cells and organisms. Biochem. J. 474:1935–1963.

Brosnan, J. T. 2001. Amino acids, then and now—a reflection on Sir Hans Krebs' contribution to nitrogen metabolism. IUBMB Life 52:265–270.

Brzostowski, H., and Z. Tański. 2006. Nutritional value of the meat of Pomeranian breed lambs and crossbreeds of Blackheaded and Texel rams. Archiv. fur. Tierzucht. 49:345–352.

Chen, G., and Y. Sui. 2018. Production, performance, slaughter characteristics, and meat quality of Ziwuling wild crossbred pigs. Trop. Anim. Health Prod. 50:365–372.
Dalle Zotte, A., R. Ricci, M. Cullere, L. Serva, S. Tenti, and G. Marchesini. 2020. Research Note: effect of chicken genotype and white striping-wooden breast condition on breast meat proximate composition and amino acid profile. Poult. Sci. 99:1797–1803.
Escobar, J., J. W. Frank, A. Suryawan, H. V. Nguyen, S. R. Kimball, L. S. Jefferson, and T. A. Davis. 2005. Physiological rise in plasma leucine stimulates muscle protein synthesis in neonatal pigs by enhancing translation initiation factor activation. Am. J. Physiol. 288:E914–E921.
Escobar, J., J. W. Frank, A. Suryawan, H. V. Nguyen, S. R. Kimball, L. S. Jefferson, and T. A. Davis. 2006. Regulation of cardiac and skeletal muscle protein synthesis by individual branched-chain amino acids in neonatal pigs. Am. J. Physiol. 290:E612–E621.
Evans, K., Z. Nasim, J. Brown, H. Butler, S. Kauser, H. Varoqui, J. D. Erickson, T. P. Herbert, and A. Bevington. 2007. Acidosis-sensing glutamine pump SNAT2 determines amino acid levels and mammalian target of rapamycin signalling to protein synthesis in L6 muscle cells. J Am Soc Nephrol. 18:1426–1436.
Gingras, A. C., B. Raught, and N. Sonenberg. 1999. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. Annu. Rev. Biochem. 68:913–963.
Gumułka, M., and K. Połtowicz. 2020. Comparison of carcass traits and meat quality of intensively reared geese from a Polish genetic resource flock to those of commercial hybrids. Poult. Sci. 99:839–847.
Guo, L., H. Tian, J. Shen, C. Zheng, S. Liu, Y. Cao, C. Cai, and J. Yao. 2018. Phenylalanine regulates initiation of digestive enzyme mRNA translation in pancreatic acinar cells and tissue segments in dairy calves. Biosci. Rep. 38:BSR20171189.
Hara, K., K. Yonezawa, Q. P. Weng, M. T. Kozlowski, C. Belham, and J. Avruch. 1998. Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. J. Biol. Chem. 273:14484–14494.
Haraf, G., J. Wołoszyn, A. Okruszek, Z. Goluch, M. Wereńska, and M. Teleszko. 2021. The protein and fat quality of thigh muscles from Polish goose varieties. Poult. Sci. 100:100992.
Heublein, S., S. Kazi, M. H. Ogmundsdottir, E. V. Attwood, S. Kala, C. A. Boyd, C. Wilson, and D. C. Goberdhan. 2010. Proton-assisted amino-acid transporters are conserved regulators of proliferation and amino-acid-dependent mTORC1 activation. Oncogene 29:4068–4079.
Hilliar, M., N. Huyen, C. K. Girish, R. Barekatain, S. Wu, and R. A. Swick. 2019. Supplementing glycine, serine, and threonine in low protein diets for meat type chickens. Poult. Sci. 98:6857–6865.
Hundal, H. S., and P. M. Taylor. 2009. Amino acid transceptors: gate keepers of nutrient exchange and regulators of nutrient signaling. Am. J. Physiol. 296:E603–E613.
Hyde, R., E. Hajduch, D. J. Powell, P. M. Taylor, and H. S. Hundal. 2005. Ceramide down-regulates System A amino acid transport and protein synthesis in rat skeletal muscle cells. FASEB J. 19:461–463.
Leeson, S. 1993. Potential of modifying poultry products. J. Appl. Poult. Res. 2:380–384.
Liu, T., Q. Mo, J. Wei, M. Zhao, J. Tang, and F. Feng. 2021. Mass spectrometry-based metabolomics to reveal chicken meat improvements by medium-chain monoglycerides supplementation: taste, fresh meat quality, and composition. Food Chem. 365:130303.
Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 $-\Delta\Delta$ C T method. Methods 25:402–408.
Lorenzo, J. M., and D. Franco. 2012. Fat effect on physico-chemical, microbial and textural changes through the manufactured of dry-cured foal sausage lipolysis, proteolysis and sensory properties. Meat Sci. 92:704–714.
Lund, M. N., M. Heinonen, C. P. Baron, and M. Estévez. 2011. Protein oxidation in muscle foods: a review. Mol. Nutr. Food Res. 55:83–95.
Nasr, M. A. F., T. Abd-Elhamid, and M. A. Hussein. 2017. Growth performance, carcass characteristics, meat quality and muscle amino-acid profile of different rabbits breeds and their crosses. Meat Sci. 134:150–157.
Nicklin, P., P. Bergman, B. Zhang, E. Triantafellow, H. Wang, B. Nyfeler, H. Yang, M. Hild, C. Kung, C. Wilson, V. E. Myer, J. P. MacKeigan, J. A. Porter, Y. K. Wang, L. C. Cantley, P. M. Finan, and L. O. Murphy. 2009. Bidirectional transport of amino acids regulates mTOR and autophagy. Cell 136:521–534.
Pearson, T., O. Wendowski, and P. P. Powell. 2021. Enhanced small neutral but not branched chain amino acid transport after epigenetic sodium coupled neutral amino acid transporter-2 (SNAT2) cDNA expression in myoblasts. J Cachexia Sarcopenia Muscle 12:811–822.
Sabow, A. B. 2020. Carcass characteristics, physicochemical attributes, and fatty acid and amino acid compositions of meat obtained from different Japanese quail strains. Trop. Anima. Health Produc. 52:131–140.
Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika 52:591–611.
Siegert, W., and M. Rodehutscord. 2019. The relevance of glycine and serine in poultry nutrition: a review. Br Poult Sci 60:579–588.
Simonová, M. P., Ľ. Chrastinová, M. Chrenková, Z. Formelová, A. Kandričáková, E. Bino, and A. Lauková. 2020. Benefits of enterocin M and sage combination on the physico-chemical traits, fatty acid, amino acid, and mineral content of rabbit meat. Probiot. Antimicrob. Proteins. 12:1235–1245.
Subramaniyan, S. A., D. R. Kang, S. A. Belal, E. S. Cho, J. H. Jung, Y. C. Jung, Y. I. Choi, and K. S. Shim. 2016. Meat quality and physicochemical trait assessments of Berkshire and commercial 3-way crossbred pigs. Korean J. Food Sci. Anim. Resour. 36:641–649.
Suenaga, R., S. Tomonaga, H. Yamane, I. Kurauchi, Y. Tsuneyoshi, H. Sato, D. M. Denbow, and M. Furuse. 2008. Intracerebroventricular injection of L-arginine induces sedative and hypnotic effects under an acute stress in neonatal chicks. Amino. Acids. 35:139–146.
Tang, X., X. Liu, and H. Liu. 2021. Effects of dietary probiotic (*Bacillus subtilis*) supplementation on carcass traits, meat quality, amino acid, and fatty acid profile of broiler chickens. Front. Vet. Sci. 8:767802.
Wang, X., L. E. Campbell, C. M. Miller, and C. G. Proud. 1998. Amino acid availability regulates p70 S6 kinase and multiple translation factors. Biochem. J. 334:261–267.
Wu, G. 2009. Amino acids: metabolism, functions, and nutrition. Amino Acids 37:1–17.
Wu, W. 2003. C. M. A.. 2003. A note on determining the p -value of Bartlett's test of homogeneity of variances. Commun. Stat. Theory Methods 32:91–101.
Xu, X., X. Chen, D. Chen, B. Yu, J. Yin, and Z. Huang. 2019. Effects of dietary apple polyphenol supplementation on carcass traits, meat quality, muscle amino acid and fatty acid composition in finishing pigs. Food Funct. 10:7426–7434.
Young, V. R., and P. L. Pellett. 1984. Amino acid composition in relation to protein nutritional quality of meat and poultry products. Am. J. Clin. Nutr. 40:737–742.
Yu, C., J. Zhang, Q. Li, X. Xiang, Z. Yang, and T. Wang. 2021. Effects of trans-anethole supplementation on serum lipid metabolism parameters, carcass characteristics, meat quality, fatty acid, and amino acid profiles of breast muscle in broiler chickens. Poult. Sci. 100:101484.
Yu, M., Z. Li, T. Rong, G. Wang, Z. Liu, W. Chen, J. Li, J. Li, and X. Ma. 2020. Different dietary starch sources alter the carcass traits, meat quality, and the profile of muscle amino acid and fatty acid in finishing pigs. J. Anim. Sci. Biotechnol. 11:78.