Effects of different breeds/strains on fatty acid composition and lipid metabolism-related genes expression in breast muscle of ducks

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ABSTRACT Fatty acid composition contributes greatly to the nutritional value of meat, and breeds/strains are important factors affecting the composition of fatty acid. Recently, few studies have focused on the fatty acid composition in breast muscle of different duck breeds. Therefore, the objective of the present study was to compare the fatty acid composition and lipid metabolism-related genes expression in breast muscle of Jian-chang duck (J), Cherry Verry duck (CV) and 3 crossbred strains (BH1, BH2 and MC) in order to compare the fatty acid composition and lipid metabolism-related genes expression in breast muscle of different duck breeds. We found that the breast muscle of J had the highest contents of C22:1(n-9) but the lowest ratios of S-omega 6 (Σω-6)/S-omega 3 (Σω-3), Σ-mono-unsaturated fatty acid (ΣMUFA)/Σ saturated fatty acid (ΣSFA) and Σ-polyunsaturated fatty acid (ΣPUFA)/ΣSFA. The ΣPUFA/ΣSFA ratio was higher in breast muscle of MBG than in that of BH2 and CV, and the contents of C22:1(n-9), ΣMUFA and ΣPUFA were higher in BH1 than in BH2 and CV. Furthermore, the mRNA levels of SCD1, FADS2, ELOVL2, and ELOVL5 were significantly higher in MBG (P < 0.05), while those of FASD1 and ACACA were significantly higher in BH1 than in BH2 and CV (P < 0.05). Principal component analysis showed that fatty acids variation exhibited extensive positive loading on principal components (PCs). Correlation analysis showed that PC1 and PC3 of BH1, as well as PC1 of MBG were correlated with the mRNA levels of ACACA and FABP3, respectively. Thus, it could be concluded that cross-breeding could optimize the composition of fatty acid in breast muscle of ducks.

Key words: breast muscle, fatty acid composition, lipid metabolism-related genes, duck

INTRODUCTION

Fatty acid is an important component of animal muscle, and its content and composition contribute greatly to the nutritional value of meat (Arshad et al., 2018; Wołoszyn et al., 2020). It has been widely reported that the content and composition of fatty acid in muscle are influenced by the rearing system, nutrition, sex, age, and breeds/strains (Peng et al., 2015; Tomazin et al., 2019; Gou et al., 2020). Among these factors, breeds/strains play an important role (Zhang et al., 2019; Huang et al., 2020).

A previous study showed that different breeds significantly affected both the contents of individual fatty acids and the values of several fatty acid indices in pork longissimus muscle (Zhang et al., 2007). Moreover, the amounts of Σ-saturated fatty acid (ΣSFA), Σ-monounsaturated fatty acid (ΣMUFA) and Σ-omega 3 (Σω-3) had significant differences in muscle of different chicken breeds (Cömert et al., 2016). Results showed that the percentage of Σ-polyunsaturated fatty acid (ΣPUFA) was significantly higher in muscle of indigenous chicken breeds than in that of crossbred chickens (Franco et al., 2012). Furthermore, the breast muscle of indigenous duck breeds had a higher Σ-omega 6 (Σω-6) proportions, higher ΣPUFA/ΣSFA ratio but lower ΣSFA (Onk et al., 2019). However, a study revealed that the higher desirable fatty acids (C18:0 + ΣMUFA + ΣPUFA) were found to be higher in crossbreed goat breeds than in indigenous goat breeds (Yalcintan et al., 2018), which suggested that the cross breeding could improve the contents of desirable fatty acids.
fatty acids. In addition, it has shown that the contents of desirable fatty acids, essential fatty acids, ΣPUFA/ΣSFA and (18:0 + 18:1)/16:0 were higher in crossbred chickens than in commercial chickens (Chen et al., 2016). These results indicated that the crossbreeding could significantly affect the content and composition of fatty acids. In recent year, several researchers have further explored the molecular mechanisms regulating meat quality. Results from Yu et al. (2013) showed that stearoyl-CoA desaturase (SCD) gene was a novel candidate gene in the regulation of PUFA deposition in fat and lean pig breeds with different meat quality. In duck breast muscle, fatty acid deposition was regulated by the interaction of genes involved in lipogenesis, lipolysis, and β-oxidation (Ding et al., 2000; Cui et al., 2020; Fan et al., 2020; Frampton et al., 2020), indicating that fatty acid deposition was a complex process in duck breast muscle. Nonghua duck is a breed with better meat quality independently bred by Sichuan Agricultural University, which has BH1, MC3 × (BGF2 × GF2)2 (MBG) and BH2 crossbred strains. However, the meat quality of these ducks has not yet been evaluated.

In the present study, Jianchang duck (J) and Cherry Valley duck (CV) were used as the control group to compare the meat quality of MBG, BH1, and BH2. J was an indigenous breed with high meat quality, and CV was a commercial breed with fast growth rate. Therefore, this study aimed to compare the fatty acid composition and the expression levels of key genes related to fatty acid metabolism in breast muscle of different breeds/strains of ducks, and to analyze the correlation between the composition of fatty acids and the expression levels of genes.

**MATERIALS AND METHODS**

**Ethics Statement**

All experimental protocols involving animal manipulation were approved by the Institutional Animal Care and Use Committee (IACUC) of Sichuan Agricultural University (Permit No. DKY-20170913).

**Animals and Sample Collection**

A total of 100 healthy 56-day-old ducks were used in this study, including Nonghua ducks (BH1, BH2, and MBG), CV and J, with 20 ducks in each breed/strain. All ducks were hatched at the same time and raised under the same condition of natural light and temperature at the Waterfowl Breeding Experimental Farm of Sichuan Agricultural University (Ya’an, Sichuan, China). All ducks were reared with the same diet in Table 1 (Sanwang Agriculture and Animal Husbandry Co., Ltd, Chengdu, China). They were provided with free access to feed and water until they were slaughtered at 56-day-old. After fasting for 12 h, 20 ducks, including 10 males and 10 females, were selected from each breed/strain and slaughtered. After exsanguination, breast muscle (pectoralis major) from the left side was rapidly collected and stored at −80°C for determination of fatty acid composition and extraction of RNA.

**Table 1.** Ingredients and nutrients composition of basal diets.

| Ingredients | Stage (15–56 d) |
|-------------|-----------------|
| Items       |                 |
| 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) | 2900         |
| 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           |

**Determination of Fatty Acid Composition and Content**

The determination method of fatty acids content was carried out according to GB 5009.168-2016 (2016). A total of 200 mg breast muscle was weighed for dilute acid hydrolysis. First, 100 mg of pyrogallic acid, a few grains of zeolite, 2 mL of 95% ethanol, and 10 mL of hydrochloric acid solution were mixed and added into a flask containing the breast muscle sample. Then, the flask was placed into a water bath for incubation at 70°C to 80°C for 40 min and shaken every 10 min. After incubation, the hydrolysate was transferred into the separating funnel and mixed with 10 mL of 95% ethanol. Subsequently, the mixture was extracted by adding a mixture containing 50 mL of diethyl ether and petroleum ether (1:1 vol/vol) into the separating funnel. The ether layer extract was collected into a 250 mL flask by shaking for 5 min and standing for 10 min. Repeating the above steps 3 times, the extract was dried in an oven at 100°C ± 5°C for 2 h. After that, the extract and 2 mL of 2% sodium hydroxide methanol solution were mixed to saponify and esterify under water bath at 85°C for 30 min, followed that 3 mL of 14% boron trifluoride methanol solution was added under water bath at 85°C for 30 min. Next, 1 mL of n-Hexane was mixed with the extraction to shake for 2 min and stand for 1 h, 100 μL of supernatant was then collected and made up to 1 mL with n-Hexane. Finally, the solution was filtered through a 0.45 mm membrane and was ready for gas chromatography analysis.
The gas chromatograph (Agilent 7890A; Agilent Technologies, Santa Clara, CA) containing a CD-2560 (100 m x 0.25 mm x 0.20 μm) capillary column and a flame ionization detector (FID) was used to quantify fatty acid methyl ester (FAME). The column oven temperature procedure was described as follows: the initial temperature was maintained at 130°C for 5 min, then a 4°C/min ramp to 240°C which was maintained for 30 min. The carrier gas used was nitrogen with a flow rate of 0.5 mL/min. The injection volume was 1 μL of each sample, and the results were normalized to the expression levels of 18S rRNA.

### Total RNA Extraction and cDNA Synthesis

Six samples, three males and three females, from each breed/strain were selected for gene expression analysis. Total RNA was extracted from each breast muscle sample using TRIzol Reagent (Invitrogen, Massachusetts, CA) according to the manufacturer’s instruction. The quality and purity of total RNA were checked by spectrophotometric absorbance at 260/280 nm and 260/230 nm, respectively. The integrity of RNA was identified by electrophoresis on a 1.5% agarose gel. The cDNA was obtained by a cDNA synthesis Kit (Takara, China) under the manufacturer's protocol with 1 μg of total RNA as a template.

### Real-time Quantitative Polymerase Chain Reaction (RT-qPCR)

The primers used for RT-qPCR were designed using the Primer Premier 5 software (Premier Biosoft International, San Francisco, CA) and were shown in Table 2. The RT-qPCR was performed in a 96-well Bio-Rad iQ5 (Bio-Rad Laboratories, Hercules, CA, USA) using a Takara ExTaq RT-PCR Kit and SYBR Green as the detection dye (Takara, China). RT-qPCR was performed in a total volume of 12.5 μL, which contained 6.25 μL of the SYBR Premix ExTaq II (Takara, China), 0.5 μL of each primer, 4.25 μL ddH₂O and 1 μL cDNA. RT-qPCR was carried out under the following condition: predenaturation at 95°C for 3 min; 40 cycles of denaturation at 95°C for 10 s; annealing at primer-specific temperature for 30 s, and extension at 72°C for 30 s, a melting curve was used to verify primers specificity. Each sample was in triplicate, and the results were normalized to the expression levels of 18S rRNA and β-actin.

### Statistical Analysis

The content and composition of fatty acid as well as the RT-qPCR data were arranged using Excel 2019 software, and were then analyzed using SAS 8.0 software (SAS Institute Inc., Cary, NC). The relative mRNA expression of target genes was calculated using the comparative Ct method (2^−ΔΔCt methods) (Livak and Schmittgen, 2001). The means of different groups were subjected to ANOVA testing, and the means were assessed for significance using the Duncan’s Multiple Range test. Results were presented as the mean ± S.E. M. Differences were considered statistically significant at P < 0.05. In addition, principal component analysis (PCA) was carried out using SPSS 26.0 (IBM, Armonk, NY) software to identify the main factors that contributed to fatty acid composition. The PC values were calculated by using equations:

\[ F_j = a_{j1}X_1 + a_{j2}X_2 + a_{j3}X_3 + \ldots + a_{jp}X_p \]
### RESULTS

#### Fatty Acid Composition and Content of Breast Muscle in Five Duck Breeds/Strains

Fatty acid composition and content of breast muscle in 5 duck breeds/strains were shown in Table 3. The results showed that the content of ΣMUFA was the highest in the 5 duck breeds/strains, followed by ΣSFA and ΣPUFA. Meanwhile, compared with other breeds/strains, the content of most fatty acids in J was higher. Specifically, C20:0, C22:0, C24:1, C22:6(n−3) and C20:2(n−6) of J were significantly higher than that of BH1 (P < 0.05). The C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0, C22:1(n−9), C24:1, ΣSFA, C18:3(n−3), C20:5(n−3), C22:6(n−3) C22:1(n−9), C18:3(n−6), C18:2(n−6), C20:2(n−6), C20:3(n−6), Σn−3, Σn−6, and ΣPUFA contents were significantly higher in breast muscle of J than that of BH2 (P < 0.05). The C17:0, C18:0, C20:0, C22:0, C24:0, C20:1, C22:1, C24:1, C22:1(n−9), C22:6(n−3), C20:2(n−6), C20:3(n−6), ΣSFA, and Σn−3 contents were significantly higher in breast muscle of J than in that of MBG (P < 0.05). The C15:0, C17:0, C18:0, C20:0, C22:0, C24:0, C20:1, C22:1, C24:1, C22:1(n−9), C22:6(n−3), C20:2(n−6), C20:3(n−6), Σn−6, Σn−3, and ΣPUFA contents were significantly higher in breast muscle of J than that of CV (P < 0.05). The comparison analysis among the remaining four duck breeds/strains except for J showed that the C17:0, C18:0, C20:0, C22:0, C24:0, C20:1, C22:1, C24:1, C22:1(n−9), C22:6(n−3), C20:2(n−6), C20:3(n−6), Σn−3, Σn−6, and ΣPUFA contents were significantly higher in breast muscle of BH1 than in that of BH2 (P < 0.05), and C18:0, C20:0, C22:0, C24:0, C20:1, C22:1, C24:1, C22:1(n−9), C22:6(n−3), C20:2(n−6), C20:3(n−6), Σn−3, Σn−6, and ΣPUFA contents were significantly higher in breast muscle of J than in that of MBG (P < 0.05). Moreover, the C14:0 content of J was significantly higher than that of BH1, BH2, MBG, and CV (P < 0.05). However, both the C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0, C20:1, C22:1, C24:1, C22:1(n−9), C22:6(n−3), C20:2(n−6), C20:3(n−6), Σn−3, and ΣPUFA/ΣSFA values were significantly higher in breast muscle of J than in that of CV (P < 0.05).
PCA of Fatty Acids in Breast Muscle

To decrease the number of variables without much loss of the data set, PCA was conducted to analyze the variance of fatty acids composition in breast muscle of 5 duck breeds/strains. As shown in Table 4, 4 orthogonal principal components (PC of BH1 [HPC] 1, HPC2, HPC3, and HPC4) accounting for 87.48% of variance in breast muscle, 4 PC of BH2 ([BPC] 1, BPC2, BPC3, and BPC4) accounting for 88.72% of variance, 3 PC of MBG ([MPC] 1, MPC2, and MPC3) accounting for 82.95% of variance, 3 PC of CV ([CPC] 1, CPC2, and CPC3) accounting for 85.48% of variance, and 4 PC of J ([JPC] 1, JPC2, JPC3, and JPC4) accounting for 84.85% of variance were generated by PCA with Kaiser’s rule of eigenvalues >1. HPC1, BPC1, MPC1, CPC1, and JPC1 had the highest eigenvalue of 9.32, 10.44, 10.49, 14.13, and 11.00, respectively. They accounted for 44.37%, 47.43%, 47.68%, 64.24%, and 50.01% of the variance, respectively. Although BH1, BH2, MBG, and J had different variance, the variance of PC1 were mainly contributed by the similar composition of fatty acids with high positive loadings of C14:0, C16:0, C17:0, C18:0, C18:1(n−9)c, C20:1, C18:2(n−6)c, C18:3(n−3) (Table 5). Notably, the CPC1 was contributed by most fatty acids except C24:0, C16:1, C18:3(n−6), C20:5(n−3). In PC2, HPC2, BPC2, MPC2, and CPC2 were contributed by SFA, MUFA, and PUFA, while JPC2 was mainly contributed by SFA except C24:1 (0.739).

Effects of Breeds/Strains on the mRNA Levels of Lipid Metabolism-related Genes in Breast Muscles of Ducks

The mRNA levels of SCD1, fatty acid desaturase 2 (FADS2), fatty acid desaturase 1 (FADS1), elongation of very long chain fatty acid 5 (ELOVL5), elongation of very long chain fatty acid 2 (ELOVL2), carnitine palmitoyltransferase 1A (CPT1A), sterol regulatory element-binding protein 1 (SREBP1), fatty acid binding protein 3 (FABP3), fatty acid transport 1 (FATP1), and acetyl-CoA carboxylase (ACACA) genes in five duck breeds/strains were shown in Figure 1. The results showed that the expression level of SCD1 was the highest in breast muscle of MBG (P < 0.05). Further analysis showed that the expression levels of SCD1 were significantly higher in CV than in BH1, BH2, and J (P < 0.05). The expression levels of SREBP1 were the highest in BH2 (P < 0.05), and were significantly higher in CV than in BH1 and J (P < 0.05). In addition, the expression levels of FADS2 were significantly higher in MBG than in BH1 and CV (P < 0.05). The expression levels of FADS1 were the highest in BH1 (P < 0.05), and were significantly higher in BH2 and CV than in MBG (P < 0.05). Moreover, the expression levels of ACACA were the highest in J (P < 0.05), and were significantly higher in J than in BH1 and CV, BH2, and MBG (P < 0.05). The expression levels of ELOVL2 were significantly higher in MBG and J than in others (P < 0.05), and were significantly higher in CV than in BH2 (P < 0.05). Notably, the expression levels of ELOVL5 were significantly higher in MBG than in BH1, BH2, CV, and J (P < 0.05). The expression levels of CPT1A, FATP1, and FABP3 were significantly higher in BH2 than in BH1, MBG, CV, and J (P < 0.05), whereas the expression levels of FATP1 were significantly higher in J than in BH1, CV, and MBG (P < 0.05). Taken together, the expression levels of ACACA were higher in J and BH1, and those of ELOVL2 were higher in MBG and J. The expression levels of SCD1, ELOVL5, and FADS2 genes were the highest in MBG, that of FADS1 was highly expressed in BH1, and the expression levels of CPT1A, FABP3, FATP1, and SREBP1 genes were the highest in BH2.

Table 4. Eigenvalues of the correlation matrix.

| Items | Eigenvalue | Variance contribution (%) | Cumulative contribution (%) |
|-------|------------|---------------------------|-----------------------------|
| BH1   | HPC1       | 9.32                      | 44.37                       | 44.37                       |
|       | HPC2       | 6.65                      | 31.69                       | 76.06                       |
|       | HPC3       | 1.21                      | 5.78                        | 81.84                       |
|       | HPC4       | 1.18                      | 5.64                        | 87.48                       |
| BH2   | BPC1       | 10.44                     | 47.43                       | 47.43                       |
|       | BPC2       | 4.83                      | 21.94                       | 69.37                       |
|       | BPC3       | 2.98                      | 13.56                       | 82.94                       |
|       | BPC4       | 1.27                      | 5.79                        | 88.72                       |
| MBG   | MPC1       | 10.49                     | 47.68                       | 47.68                       |
|       | MPC2       | 6.02                      | 27.36                       | 75.04                       |
|       | MPC3       | 1.74                      | 7.91                        | 82.95                       |
| CV    | CPC1       | 14.13                     | 64.24                       | 64.24                       |
|       | CPC2       | 3.61                      | 16.40                       | 80.64                       |
|       | CPC3       | 1.06                      | 4.84                        | 85.48                       |
| J     | JPC1       | 11.00                     | 50.01                       | 50.01                       |
|       | JPC2       | 4.57                      | 20.78                       | 70.80                       |
|       | JPC3       | 1.56                      | 7.08                        | 77.87                       |
|       | JPC4       | 1.54                      | 6.98                        | 84.85                       |

1HPC, principal components of BH1; BPC, principal components of BH2; MPC, principal components of MBG; CPC, principal components of CV; JPC, principal components of J. Abbreviation: CV: Cherry Valley duck; J: Jianchang duck; MBG: MC × (BGF2 × GF2)).
Table 5. Statistical loadings of variables from PCA of the five different duck breeds/strains.

| Items   | BH1       | BH2       | MBG       | CV        | JPC       |
|---------|-----------|-----------|-----------|-----------|-----------|
| C14:0   | 0.957     | 0.916     | 0.940     | 0.839     | 0.987     |
| C15:0   | 0.174     | 0.831     | 0.929     | 0.867     | 0.876     |
| C16:0   | 0.983     | 0.971     | 0.981     | 0.981     | 0.999     |
| C17:0   | 0.933     | 0.916     | 0.929     | 0.970     | 0.903     |
| C18:0   | 0.879     | 0.769     | 0.795     | 0.795     | 0.868     |
| C20:0   | 0.673     | 0.489     | 0.431     | 0.868     | 0.239     |
| C22:0   | 0.182     | 0.091     | -0.230    | 0.725     | -0.147    |
| C24:0   | 0.211     | -0.278    | -0.052    | 0.578     | -0.531    |
| C14:1   | -         | 0.527     | 0.811     | 0.720     | 0.904     |
| C16:1   | 0.862     | 0.932     | 0.925     | 0.633     | 0.974     |
| C18:1(n-9)c | 0.931   | 0.981     | 0.931     | 0.985     | 0.983     |
| C20:1   | 0.889     | 0.938     | 0.927     | 0.857     | 0.932     |
| C22:1(n-9) | 0.896   | 0.278     | -0.015    | 0.842     | -0.503    |
| C24:1   | 0.319     | -0.346    | -0.141    | 0.904     | -0.344    |
| C18:2(n-6)c | 0.982   | 0.963     | 0.979     | 0.934     | 0.982     |
| C18:3(n-6) | 0.697   | 0.797     | 0.742     | 0.945     | 0.975     |
| C20:2(n-6) | 0.531   | 0.435     | 0.578     | 0.788     | 0.272     |
| C20:3(n-6) | 0.177   | 0.336     | 0.378     | 0.730     | 0.156     |
| C20:4(n-6) | 0.224   | 0.892     | 0.415     | 0.831     | 0.261     |
| C18:3(n-3) | 0.927   | 0.967     | 0.934     | 0.803     | 0.958     |
| C20:5(n-3) | -0.218  | 0.285     | 0.438     | 0.666     | -0.333    |
| C22:6(n-3) | -0.026  | 0.114     | 0.013     | 0.729     | -0.512    |

1The loading displayed in boldface were variables contributed greatly to the principal components.
2HPC, principal components of BH1; MPC, principal components of BH2; MBG, principal components of MBG; CPC, principal components of CV; JPC, principal components of J. Abbreviation: PCA: principal component analysis.
Correlation of PC with Fatty Acids Composition Parameters and Genes Expression Levels

The scores of PC were further calculated for correlation analysis with fatty acids composition parameters and genes expression. The factor score coefficients used for calculating the scores of each PC was showed in Table 6. Subsequently, Pearson’s correlation analysis was performed to determine the relationships of PC with fatty acids composition parameters and lipid metabolism-related genes in breast muscle. As shown in Table 7, $\Sigma$MUFA/$\Sigma$SFA, $\Sigma$PUFA/$\Sigma$SFA, and $\Sigma$n–6/$\Sigma$n–3 were positively correlated with PC1 in five duck breeds/strains. Further analysis showed that fatty acid composition and content were significantly different among 5 duck breeds/strains, which confirmed the results from a previous study that the breeds/strains had a significant effect on the contents of fatty acids (Ding et al., 2021).

In the present study, the contents of most individual fatty acids were significantly higher in breast muscle of J, which was determined by its indigenous lineage. Tu et al. (2021) showed that indigenous breeds had higher unsaturated fatty acids contents than crossed breeds. However, indigenous breeds had lower reproductive and growth rate (Gaur et al., 2018), which is not conducive to commercial breeding. CV was a fast-growing commercial duck but showed the lowest fatty acid contents in the present study. Comparison analysis showed that BH1 and MBG had the similar fatty acid composition to J. For example, PUFAs ($n$–3, $n$–6) and C22:1($n$–9) content as well as $\Sigma$PUFA/$\Sigma$SFA and $\Sigma$MUFA/$\Sigma$SFA ratio were higher, while the $\Sigma$n–6/$\Sigma$n–3 ratio was lower in breast muscle of MBG and BH1. Previous studies have shown that the lower $\Sigma$n–6/$\Sigma$n–3 ratio was more beneficial to human health (de Bus et al., 2019; Rymer and Givens, 2005), and the contents of PUFAs ($n$–3, $n$–6) enhanced the meat flavor (Cui et al., 2015). MBG and BH1 had a lower $\Sigma$n–6/$\Sigma$n–3 value but higher contents of PUFAs ($n$–3, $n$–6), which may be determined by different breeds/strains, environment, or diet. Further analysis showed that fatty acid composition and content were significantly different among 5 duck breeds/strains, which confirmed the results from a previous study that the breeds/strains had a significant effect on the contents of fatty acids.

DISCUSSION

The effects of fatty acids on meat quality were mainly dependent on the contents of unsaturated fatty acids (Hoa et al., 2020; Kouba et al., 2008). In this study, the content of $\Sigma$MUFA was the highest in the 5 duck breeds/strains, while previous studies in other duck breeds showed that $\Sigma$SFA was the highest (Chen et al., 2016; Franco et al., 2012), which may be determined by different breeds/strains, environment, or diet. Figure 1. Effects of breeds/strains on the expression levels of lipid metabolism-related genes in duck breast muscle. **a-d** Indicated a significance ($P < 0.05$) of Duncan’s multiple-rang tests among BH1, BH2, MBG, CV and J in breast muscle. Abbreviations: CV, Cherry Valley duck; J, Jianchang duck; MBG, MC££GF2; BH1, BH2, MBG, CV, J. Genes: acetyl-CoA carboxylase, ACACA; carnitine palmitoyltransferase 1A, CPT1A; elongation of very long chain fatty acid 5, ELOVL5; fatty acid binding protein 3, FABP3; fatty acid desaturase 1, FADS1; fatty acid desaturase 2, FADS2; fatty acid transport 1, FATP1; stearoyl-CoA desaturase, SCD1 and sterol regulatory element-binding protein 1, SREBP1.
Table 6. Factor score coefficients from PCA of the five different duck breeds/strains.

| Items       | BH1 | BH2 | MBG | CV  | J  |
|-------------|-----|-----|-----|-----|----|
|             | HPC | HPC | HPC | HPC | JPC|
| C14:0       | 0.103 | -0.023 | -0.124 | 0.031 |    |
| C15:0       | 0.019 | -0.059 | 0.533 | 0.365 |    |
| C16:0       | 0.105 | -0.018 | -0.002 | -0.013 |    |
| C17:0       | 0.100 | -0.021 | -0.106 | 0.075 |    |
| C18:0       | 0.094 | 0.062 | 0.053 | 0.050 |    |
| C20:0       | 0.072 | 0.095 | 0.067 | -0.106 |    |
| C22:0       | 0.020 | 0.130 | 0.105 | -0.054 |    |
| C24:0       | 0.023 | 0.134 | 0.024 | -0.180 |    |
| C14:1       | -   | -   | -   | -   | -  |
| C16:1       | 0.093 | -0.054 | -0.122 | -0.038 |    |
| C18:1(n-9)c | 0.100 | -0.047 | 0.040 | -0.108 |    |
| C20:1       | 0.095 | -0.042 | 0.181 | -0.145 |    |
| C22:1(n-9)  | -0.010 | 0.138 | 0.069 | -0.193 |    |
| C24:1       | 0.034 | 0.125 | 0.076 | -0.203 |    |
| C18:2(n-6)c | 0.105 | -0.012 | 0.013 | 0.020 |    |
| C18:3(n-6)  | 0.075 | -0.032 | -0.012 | 0.383 |    |
| C20:2(n-6)  | 0.057 | 0.102 | -0.016 | 0.208 |    |
| C20:3(n-6)  | 0.019 | 0.122 | 0.062 | 0.181 |    |
| C20:4(n-6)  | 0.024 | 0.108 | -0.288 | -0.283 |    |
| C22:3(n-3)  | 0.099 | -0.052 | -0.064 | -0.028 |    |
| C22:5(n-3)  | -0.023 | 0.065 | -0.478 | 0.449 |    |
| C22:6(n-3)  | -0.003 | 0.106 | 0.315 | 0.097 |    |

1HPC, principal components of BH1; BPC, principal components of BH2; MPC, principal components of MBG; CPC, principal components of CV; JPC, principal components of J. Abbreviation: PCA: principal component analysis.
Table 7. The Pearson correlation analysis of principal composition, fatty acids composition, and genes expression

| Items | CV | HPC1 | HPC2 | HPC3 | HPC4 | BPC1 | BPC2 | BPC3 | BPC4 | MPC1 | MPC2 | MPC3 | CPC1 | CPC2 | CPC3 | JPC1 | JPC2 | JPC3 | JPC4 |
|-------|----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Genes | | | | | | | | | | | | | | | | | | | | |
| ACACA | 0.828* | 0.869* | 0.753 | 0.703 | 0.699 | 0.941 | 0.716 | 0.716 | 0.703 | 0.693 | 0.703 | 0.699 | 0.941 | 0.716 | 0.716 | 0.703 | 0.693 | 0.703 |
| SCD1  | 0.169 | 0.170 | 0.171 | 0.172 | 0.173 | 0.174 | 0.175 | 0.176 | 0.177 | 0.178 | 0.179 | 0.180 | 0.181 | 0.182 | 0.183 | 0.184 | 0.185 | 0.186 |
| FADS1  | 0.210 | 0.211 | 0.212 | 0.213 | 0.214 | 0.215 | 0.216 | 0.217 | 0.218 | 0.219 | 0.220 | 0.221 | 0.222 | 0.223 | 0.224 | 0.225 | 0.226 | 0.227 |
| FADS2  | 0.240 | 0.241 | 0.242 | 0.243 | 0.244 | 0.245 | 0.246 | 0.247 | 0.248 | 0.249 | 0.250 | 0.251 | 0.252 | 0.253 | 0.254 | 0.255 | 0.256 | 0.257 |
| ELOVL2 | 0.260 | 0.261 | 0.262 | 0.263 | 0.264 | 0.265 | 0.266 | 0.267 | 0.268 | 0.269 | 0.270 | 0.271 | 0.272 | 0.273 | 0.274 | 0.275 | 0.276 | 0.277 |
| ELOVL5 | 0.280 | 0.281 | 0.282 | 0.283 | 0.284 | 0.285 | 0.286 | 0.287 | 0.288 | 0.289 | 0.290 | 0.291 | 0.292 | 0.293 | 0.294 | 0.295 | 0.296 | 0.297 |
| CPT1A | 0.290 | 0.291 | 0.292 | 0.293 | 0.294 | 0.295 | 0.296 | 0.297 | 0.298 | 0.299 | 0.300 | 0.301 | 0.302 | 0.303 | 0.304 | 0.305 | 0.306 | 0.307 |

which suggested that the breast muscle of MBG and BH1 might have better fatty acid composition. Other studies showed that the high content of MUFA could increase the palatability of meat, while SFA could increase the risk of cardiovascular diseases (Janiszewski et al., 2018; Jiang et al., 2013; Temple, 2018). The C22:1(n−9) was one of the most abundant MUFA among 5 duck breeds/strains. Moreover, coefficient of the factor score analysis showed that PC2 of both BH1 and MBG were mainly contributed by C22:1(n−9), while PC1 of each breed/strain was contributed by most of fatty acids. Notably, the PC1 and PC2, in BH1 and MBG, were extremely significantly correlated with ΣPUFA/ΣSFA and Σn−6/Σn−3. These results suggested that the fatty acids compositions and individual fatty acids contents might be optimized in breast muscle of MBG and BH1 compared with CV and BH2.

To explore the molecular mechanism underlying the different fatty acid compositions and contents among different duck breeds/strains, the expression levels of ACACA, SCD1, FADS1, FADS2, ELOVL5, ELOVL2, FATP1, FABP3, and CPT1A were further detected. Among them, ACACA was considered as a pivotal enzyme in de novo synthesis of fatty acids (Liu et al., 2019), and the mRNA levels of ACACA were observed to be higher in breast muscle of J and in that of BH1. Our results demonstrated that the de novo synthesis of fatty acids is better in indigenous and crossed breeds. Furthermore, analysis showed that some important genes involved in transport, elongation, desaturation and β-oxidation of fatty acids were also significant differences in different duck breeds/strains. Among these genes, SCD1, FADS1, and FADS2 were key enzymes involved in catalyzing the desaturation of C16:0 and C18:0 into C16:1 and C18:1 (Glaser et al., 2010; Zhu Cai-Ye et al., 2013), and the mRNA expression levels of these genes were significantly higher in breast muscle of J, MBG and BH1 than in those of CV, which indicated that the process of fatty acid desaturation in breast muscle of ducks could be improved by crossbreeding. Result showed that C18:2(n−6)c and C18:3(n−3) could produce long-chain polyunsaturated fatty acids (LC-PUFA) by D-5 and D-6 desaturases and elongates which were driven by ELOVL2 and ELOVL5 (Castro et al., 2016). Our results showed that the mRNA levels of ELOVL2 and ELOVL5 were the highest in MBG, which suggested that the better fatty acid composition in MBG was related to the elongation and desaturation of fatty acids. In addition, SREBP1, CPT1A, FATP1, and FABP3 exhibited the highest expression levels in BH2. Previous studies showed that FATP1 was involved in transport of fatty acids from the capillary to cytoplasm, while FABP3 was involved in transport of fatty acids from the cytoplasm to organelle membrane (Gerbens et al., 1999; Zhang et al., 2013). CPT1A played an important role in β-oxidation process (Nakamura et al., 2014). Moreover, SREBP1 could activate fatty acid transport and oxidation processes (Wang et al., 2017; Xie et al., 2017), and FATP1, FABP3, and CPT1A were regulated by
SREBP1 (Huang et al., 2021; Rottiers et al., 2011; Xu et al., 2018). These results indicated that the transport and oxidation of fatty acids in breast muscle of BH2 might be more active.

The correlations of PC with fatty acids composition parameters and genes expression levels were further carried out. Our results showed that the ΣMUFA/ΣSFA, ΣPUFA/ΣSFA, and Σn−6/Σn−3 ratios were positively correlated with PC1 in 5 duck breeds/strains. In addition, the PCs described the most of variance HPC1, JPC1, and HPC3 were positively correlated with ACACA, which suggested that the higher content of fatty acids in J and BH1 might be associated with the synthesis of fatty acids. However, the BPC2 and BPC4 were negatively correlated with ACACA and ELOVL5, respectively. BPC1 and BPC3 were positively correlated with FABP3. These results suggested that the lower content of fatty acids in BH1 was related to the strong transport and oxidation ability of fatty acids in breast muscle. Additionally, MPC2 was positively correlated with FABP3, suggesting that the fatty acids transport process was regulated by FABP3 in breast muscle of MBG.

In conclusion, among the 5 duck breeds/strains, the fatty acid composition in breast muscle of BH1 and MBG was more similar to that in J, and was better than that in BH2 and CV. The better fatty acid composition of breast muscle of MBG and BH1 were contributed by the increased contents of unsaturated fatty acids, which was closely related to the increased expression levels of SCDO1, FADS2, ELOVL2, and ELOVL5 genes in breast muscle of MBG but FADS1 and ACACA in breast muscle of BH1. Therefore, MBG and BH1 had the higher ratios of ΣPUFA/ΣSFA and ΣMUFA/ΣSFA but lower Σn−6/Σn−3 ratio. Although further studies are required to elucidate the underlying mechanisms, the way of crossbreeding might be helpful to optimize the fatty acid composition in breast muscle of duck.

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DISCLOSURES

The author(s) declare(s) no conflicts of interest.

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