

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

Xin Zhang,<sup>1</sup> Yan Deng,<sup>1</sup> Jiaming Ma, Shenqiang Hu, Jiwei Hu, Bo Hu, Hehe Liu, Liang Li, Hua He, and Jiwen Wang<sup>2</sup>

*Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province, Chengdu Campus, Sichuan Agricultural University, Wenjiang District, 611130, Chengdu, Sichuan, China*

**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 Jianchang duck (**J**), Cherry Verry duck (**CV**) and 3 crossbred strains (BH1, BH2 and MC♂ × (BGF2♂ × GF2♀)♀ (MBG)). Our results showed that the breast muscle of J had the highest contents of C22:1(n-9) but the lowest ratios of  $\Sigma$ -omega 6 ( $\Sigma n-6$ )/ $\Sigma$ -omega 3 ( $\Sigma n-3$ ),  $\Sigma$ -mono-unsaturated fatty acid ( $\Sigma$ MUFA)/ $\Sigma$ -saturated fatty acid ( $\Sigma$ SFA) and  $\Sigma$ -polyunsaturated fatty acid ( $\Sigma$ PUFA)/ $\Sigma$ SFA. The  $\Sigma$ PUFA/ $\Sigma$ SFA ratio was higher in breast muscle of MBG than in that of BH2 and CV, and the contents of C22:1(n-9),  $\Sigma$ MUFA and

$\Sigma$ PUFA 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 the breast muscles of MBG and BH1 have better fatty acid composition, which was closely related to the increased expression levels of *SCD1*, *FADS2*, *ELOVL2*, and *ELOVL5* genes in MBG but *FADS1* and *ACACA* in BH1. Moreover, these results also showed that crossbreeding could optimize the composition of fatty acid in breast muscle of ducks.

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

2022 Poultry Science 101:101813

<https://doi.org/10.1016/j.psj.2022.101813>

## 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; Tomažin 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  $\Sigma$ -saturated fatty acid ( $\Sigma$ SFA),  $\Sigma$ -monounsaturated fatty acid ( $\Sigma$ MUFA) and  $\Sigma$ -omega 3 ( $\Sigma n-3$ ) had significant differences in muscle of different chicken breeds (Cömert et al., 2016). Results showed that the percentage of  $\Sigma$ -polyunsaturated fatty acid ( $\Sigma$ PUFA) 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  $\Sigma$ -omega 6 ( $\Sigma n-6$ ) proportions, higher  $\Sigma$ PUFA/ $\Sigma$ SFA ratio but lower  $\Sigma$ SFA (Onk et al., 2019). However, a study revealed that the higher desirable fatty acids (C18:0 +  $\Sigma$ MUFA +  $\Sigma$ PUFA) were found to be higher in crossbred goat breeds than in indigenous goat breeds (Yalcintan et al., 2018), which suggested that the cross breeding could improve the contents of desirable

© 2022 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received December 4, 2021.

Accepted February 22, 2022.

<sup>1</sup>These authors have contributed equally to this work.

<sup>2</sup>Corresponding author: [wjw2886166@163.com](mailto:wjw2886166@163.com)

fatty acids. In addition, it has shown that the contents of desirable fatty acids, essential fatty acids,  $\Sigma$ PUFA/ $\Sigma$ SFA 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  $\beta$ -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, MC $\delta$   $\times$  (BGF2 $\delta$   $\times$  GF2 $\delta$ ) $\delta$  (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

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

Items	Stage (15–56 d)
<b>Ingredients</b>	
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
<b>Nutrients</b>	
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

collected and stored at  $-80^{\circ}\text{C}$  for determination of fatty acid composition and extraction of RNA.

### 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 pyrogalllic 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  $70^{\circ}\text{C}$  to  $80^{\circ}\text{C}$  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  $100^{\circ}\text{C} \pm 5^{\circ}\text{C}$  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  $85^{\circ}\text{C}$  for 30 min, followed that 3 mL of 14% boron trifluoride methanol solution was added under water bath at  $85^{\circ}\text{C}$  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  $\mu\text{L}$  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.

**Table 2.** Primers used for RT-qPCR.

Primer name	Primer sequence (5'-3')	GenBank	TM (°C)
<i>ELOVL2</i> -F	CGGGATTCCAGGGTTAGAGG	XM_038175034.1	61.8
<i>ELOVL2</i> -R	CCCAAGGTTATATACAATGAGGTG		
<i>FADS2</i> -F	AGGGGAACCCAGCCAAGAT	XM_013103963.4	61.0
<i>FADS2</i> -R	GGTGCCGAAGTATGAAACCATT		
<i>ACACA</i> -F	TCTCCCAACCCGCTAAACC	XM_038165887.1	59.9
<i>ACACA</i> -R	TATCCCTCCGAAACGAGTAAC		
<i>SREBP1</i> -F	CGAGTACATCCGCTTCCTGC	AY613441.1	61.2
<i>SREBP1</i> -R	TGAGGGACTTGCTCTTCTGC		
<i>FATP1</i> -F	CGGCGAGTTCTACGGAGC	XM_005018222	58.3
<i>FATP1</i> -R	GTAAGCAATCTTCTGTGGGGTG		
<i>FABP3</i> -F	GGGCTGACCAAACCCACC	XM_027443958.1	68.1
<i>FABP3</i> -R	GCTCCCGCACTAGCGATGTCTC		
<i>SCD1</i> -F	GCTTCTTCATTCCAGCCATCC	KF185111.1	63.6
<i>SCD1</i> -R	CCATCTCCAGTCCGCATTTTCC		
<i>FADS1</i> -F	AGATTTCGGTGAATCCG	XM_027459434.2	56.0
<i>FADS1</i> -R	AAGCTGCAATATCCAACAAC		
<i>ELOVL5</i> -F	TTGGCTTGGACCCAGAGAC	NM_001310419.1	59.6
<i>ELOVL5</i> -R	ACAGGGAAAGCAGCGTGAGT		
<i>CPT1A</i> -F	CAGATGTTATGACAGGTGGTTTG	AM883113.1	58.4
<i>CPT1A</i> -R	TCAGTTGCCATTACATTCTCCC		
<i>18S</i> -F	TTGGTGGAGCGATTTGTC	XR_005262538.1	55.0
<i>18S</i> -R	ATCTCGGGTGGCTGAACC		
$\beta$ - <i>ACTIN</i> -F	GCTATGTCGCCCTGGATTTTC	EF667345.1	61.0
$\beta$ - <i>ACTIN</i> -R	CACAGGACTCCATACCCAAGAA		

The gas chromatograph (Agilent 7890A; Agilent technologies, Santa Clara, CA) containing a CD-2560 (100 m  $\times$  0.25 mm  $\times$  0.20  $\mu$ m) 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  $\mu$ L at a split ratio of 10:1, and the injector and FID detector temperature were kept at 250°C. Identification of FAMES was performed by comparing the retention times with authentic standards (FAME mix 35 components). The results were expressed as mg/kg of FAMES identified.

### 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  $\mu$ g 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  $\mu$ L, which contained 6.25  $\mu$ L of the SYBR Premix ExTaq II (Takara, China), 0.5  $\mu$ L of each primer, 4.25  $\mu$ L ddH<sub>2</sub>O and 1  $\mu$ L cDNA. RT-qPCR was carried out under the following condition: pre-denaturation 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  $\beta$ -*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 ( $2^{-\Delta\Delta C_t}$  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  $\pm$  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:

$$F_j = a_{j1}X_1 + a_{j2}X_2 + a_{j3}X_3 + \dots + a_{jp}X_p$$

**Table 3.** Fatty acid composition (g/10kg) in breast muscle of five duck breeds/strains.

Items <sup>1</sup>	Breeds/Strains				
	BH1	BH2	MBG	CV	J
C12:0	-	-	-	0.062 ± 0.006	0.070 ± 0.006
C14:0	0.856 ± 0.083 <sup>b</sup>	0.805 ± 0.065 <sup>b</sup>	1.197 ± 0.083 <sup>a</sup>	0.926 ± 0.066 <sup>b</sup>	1.030 ± 0.090 <sup>ab</sup>
C15:0	0.101 ± 0.009 <sup>abc</sup>	0.082 ± 0.006 <sup>c</sup>	0.105 ± 0.007 <sup>ab</sup>	0.094 ± 0.007 <sup>bc</sup>	0.121 ± 0.008 <sup>a</sup>
C16:0	26.059 ± 1.904 <sup>a</sup>	19.941 ± 1.287 <sup>b</sup>	23.480 ± 1.436 <sup>ab</sup>	22.457 ± 1.471 <sup>ab</sup>	27.478 ± 1.945 <sup>a</sup>
C17:0	0.306 ± 0.018 <sup>ab</sup>	0.214 ± 0.013 <sup>c</sup>	0.289 ± 0.015 <sup>b</sup>	0.274 ± 0.018 <sup>b</sup>	0.352 ± 0.019 <sup>a</sup>
C18:0	27.871 ± 1.201 <sup>a</sup>	21.656 ± 0.882 <sup>b</sup>	22.365 ± 0.864 <sup>b</sup>	23.117 ± 1.375 <sup>b</sup>	30.625 ± 1.058 <sup>a</sup>
C20:0	0.672 ± 0.033 <sup>b</sup>	0.615 ± 0.026 <sup>b</sup>	0.619 ± 0.023 <sup>b</sup>	0.698 ± 0.044 <sup>b</sup>	0.964 ± 0.024 <sup>a</sup>
C22:0	0.426 ± 0.024 <sup>b</sup>	0.343 ± 0.021 <sup>c</sup>	0.326 ± 0.015 <sup>c</sup>	0.396 ± 0.030 <sup>bc</sup>	0.646 ± 0.026 <sup>a</sup>
C23:0	-	-	-	-	0.693 ± 0.021
C24:0	0.340 ± 0.022 <sup>ab</sup>	0.299 ± 0.013 <sup>b</sup>	0.296 ± 0.019 <sup>b</sup>	0.381 ± 0.026 <sup>b</sup>	0.489 ± 0.014 <sup>a</sup>
ΣSFA	56.599 ± 3.095 <sup>ab</sup>	43.928 ± 2.177 <sup>c</sup>	48.675 ± 2.280 <sup>bc</sup>	48.340 ± 2.911 <sup>bc</sup>	62.182 ± 3.096 <sup>a</sup>
C14:1	-	0.059 ± 0.004 <sup>b</sup>	0.093 ± 0.008 <sup>a</sup>	0.079 ± 0.008 <sup>ab</sup>	0.083 ± 0.008 <sup>ab</sup>
C16:1	3.136 ± 0.388	3.087 ± 0.305	4.091 ± 0.324	3.785 ± 0.320	3.821 ± 0.449
C18:1(n-9)c	69.610 ± 7.803	62.925 ± 5.723	59.605 ± 5.584	58.625 ± 4.626	70.114 ± 8.287
C20:1	0.683 ± 0.075 <sup>ab</sup>	0.646 ± 0.054 <sup>ab</sup>	0.582 ± 0.052 <sup>b</sup>	0.575 ± 0.043 <sup>b</sup>	0.793 ± 0.079 <sup>a</sup>
C22:1(n-9)	19.515 ± 0.880 <sup>ab</sup>	12.816 ± 0.384 <sup>d</sup>	17.788 ± 0.691 <sup>bc</sup>	15.799 ± 0.959 <sup>c</sup>	20.642 ± 0.549 <sup>a</sup>
C24:1	0.318 ± 0.020 <sup>b</sup>	0.305 ± 0.018 <sup>b</sup>	0.289 ± 0.015 <sup>b</sup>	0.341 ± 0.027 <sup>b</sup>	0.521 ± 0.017 <sup>a</sup>
ΣMUFA	93.260 ± 7.910	79.167 ± 6.190	82.438 ± 5.804	79.194 ± 5.437	95.948 ± 8.540
C18:2(n-6)c	40.458 ± 2.871 <sup>ab</sup>	31.066 ± 1.975 <sup>c</sup>	37.458 ± 2.179 <sup>abc</sup>	34.411 ± 2.051 <sup>bc</sup>	43.130 ± 3.106 <sup>a</sup>
C18:3(n-6)	0.129 ± 0.011 <sup>ab</sup>	0.104 ± 0.008 <sup>b</sup>	0.142 ± 0.010 <sup>a</sup>	0.153 ± 0.016 <sup>a</sup>	0.158 ± 0.013 <sup>a</sup>
C20:2(n-6)	0.862 ± 0.037 <sup>b</sup>	0.619 ± 0.025 <sup>c</sup>	0.914 ± 0.044 <sup>b</sup>	0.814 ± 0.069 <sup>b</sup>	1.170 ± 0.042 <sup>a</sup>
C20:3(n-6)	1.261 ± 0.051 <sup>a</sup>	1.071 ± 0.045 <sup>b</sup>	1.069 ± 0.071 <sup>b</sup>	1.374 ± 0.082 <sup>a</sup>	1.338 ± 0.057 <sup>a</sup>
C20:4(n-6)	0.459 ± 0.027	1.042 ± 0.765	0.261 ± 0.016	0.313 ± 0.026	0.300 ± 0.012
Σn-6	43.167 ± 2.905 <sup>ab</sup>	33.892 ± 2.038 <sup>c</sup>	39.844 ± 2.212 <sup>abc</sup>	36.948 ± 2.189 <sup>bc</sup>	46.086 ± 3.143 <sup>a</sup>
C18:3(n-3)	0.915 ± 0.104 <sup>ab</sup>	0.716 ± 0.069 <sup>b</sup>	1.003 ± 0.080 <sup>a</sup>	0.802 ± 0.066 <sup>ab</sup>	1.009 ± 0.099 <sup>a</sup>
C20:5(n-3)	0.178 ± 0.013 <sup>ab</sup>	0.140 ± 0.008 <sup>c</sup>	0.166 ± 0.012 <sup>bc</sup>	0.204 ± 0.013 <sup>a</sup>	0.191 ± 0.013 <sup>ab</sup>
C22:6(n-3)	1.065 ± 0.049 <sup>b</sup>	0.788 ± 0.034 <sup>d</sup>	0.927 ± 0.045 <sup>c</sup>	0.747 ± 0.050 <sup>d</sup>	1.233 ± 0.044 <sup>a</sup>
Σn-3	2.157 ± 0.101 <sup>ab</sup>	1.637 ± 0.080 <sup>c</sup>	2.095 ± 0.093 <sup>b</sup>	1.743 ± 0.103 <sup>c</sup>	2.423 ± 0.087 <sup>a</sup>
ΣPUFA	45.324 ± 3.000 <sup>ab</sup>	35.529 ± 2.109 <sup>c</sup>	41.939 ± 2.291 <sup>abc</sup>	38.691 ± 2.288 <sup>bc</sup>	48.509 ± 3.218 <sup>a</sup>
ΣMUFA/ΣSFA	1.606 ± 0.062 <sup>ab</sup>	1.762 ± 0.065 <sup>a</sup>	1.663 ± 0.050 <sup>ab</sup>	1.628 ± 0.043 <sup>ab</sup>	1.489 ± 0.065 <sup>b</sup>
ΣPUFA/ΣSFA	0.792 ± 0.014 <sup>b</sup>	0.804 ± 0.023 <sup>b</sup>	0.857 ± 0.015 <sup>a</sup>	0.801 ± 0.010 <sup>b</sup>	0.767 ± 0.016 <sup>b</sup>
Σn-6/Σn-3	19.735 ± 0.538 <sup>abc</sup>	20.617 ± 0.595 <sup>ab</sup>	18.978 ± 0.562 <sup>bc</sup>	21.239 ± 0.411 <sup>a</sup>	18.689 ± 0.794 <sup>c</sup>

<sup>a-d</sup>Indicated a significance ( $P < 0.05$ ) of Duncan's multiple-rang tests among BH1, BH2, MBG, CV and J in breast muscle.

<sup>1</sup>The items displayed in the table were selected from 35 kinds of fatty acids with detectable rates higher than 30%, otherwise were not shown in the table or displayed in "-". Abbreviations: CV: Cherry Valley duck; J: Jianchang duck; MBG: MC♂ × (BGF2♂ × GF2♀)♀; MUFA: monounsaturated fatty acid; n-3: omega 3; n-6: omega 6; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acid.

$j = 1, 2, 3, \dots, k; F_j$ , the scores of PC;  $aj_1 \sim aj_p$ , the factor score coefficients of PC on variables;  $X_1 \sim X_p$ , the fatty acids profiles of each sample;  $j$  represented the number of PC;  $p$  represented the number of variables. Subsequently, the Pearson's correlation was calculated between principal component value and fatty acid composition as well as genes expression.

## 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)c, 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 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), C18:2(n-6)c, C20:2(n-6), Σn-6, Σn-3, and ΣPUFA contents were significantly higher in breast muscle of J than in that of CV ( $P < 0.05$ ). However, the values of both Σn-6/Σn-3 and ΣMUFA/ΣSFA were the lowest in breast muscle of J ( $P < 0.05$ ).

Comparison analysis among the remaining four duck breeds/strains except for J showed that the C16:0, C18:0, C22:0, C22:1(n-9), C20:5(n-3), C22:6(n-3), C18:2(n-6)c, 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, C22:0, C22:6(n-3), C20:3(n-6) contents were significantly higher in breast muscle of BH1 than in that of MBG ( $P < 0.05$ ). However, both the C14:0 content and the ΣPUFA/ΣSFA value were the highest in breast muscle of MBG ( $P < 0.05$ ). Moreover, the C14:0, C15:0, C17:0, C14:1, C22:1(n-9), C18:3(n-3), C22:6(n-3), C18:3(n-6), C20:2(n-6), Σn-3, and ΣPUFA/ΣSFA values were significantly higher in breast muscle of



MBG than in that of BH2 ( $P < 0.05$ ), and C14:0, C22:6 (n-3),  $\Sigma n-3$ , and  $\Sigma$ PUFA/ $\Sigma$ SFA of MBG were also significantly higher than that of CV ( $P < 0.05$ ). The C18:0, C22:1(n-9), and  $\Sigma n-3$  contents were significantly higher in breast muscle of BH1 than in that of CV ( $P < 0.05$ ), whereas C20:5(n-3), C20:3(n-6), and  $\Sigma n-6/\Sigma n-3$  were significantly higher in breast muscle of CV than in that of MBG ( $P < 0.05$ ). Comparison analysis between BH2 and CV showed that C17:0, C20:5(n-3), C18:3(n-6), C20:2(n-6), and C20:3(n-6) contents were significantly higher in breast muscle of CV than in that of BH2 ( $P < 0.05$ ). Taken together, the contents of total fatty acids were the highest in breast muscle of J, followed by BH1, MBG, BH2, and CV.

### 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, C16:1, 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 and BH1 than in 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.

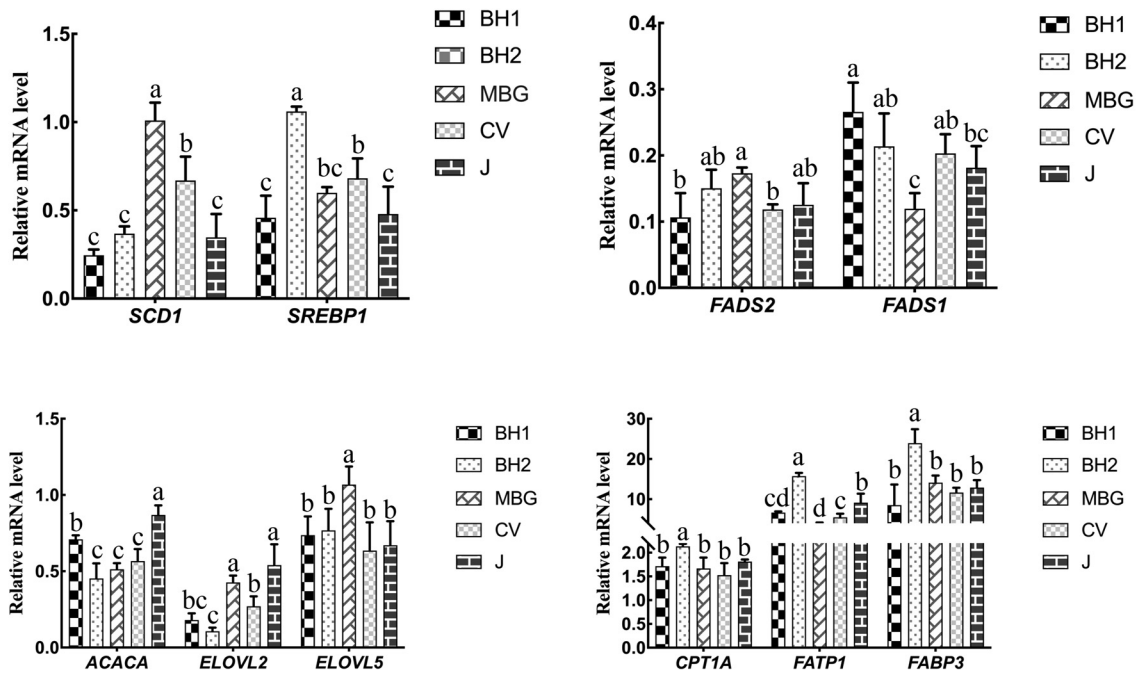
Items <sup>1</sup>	Eigenvalue	Variance contribution (%)	Cumulative contribution (%)
<b>BH1</b>			
HPC1	9.32	44.37	44.37
HPC2	6.65	31.69	76.06
HPC3	1.21	5.78	81.84
HPC4	1.18	5.64	87.48
<b>BH2</b>			
BPC1	10.44	47.43	47.43
BPC2	4.83	21.94	69.37
BPC3	2.98	13.56	82.94
BPC4	1.27	5.79	88.72
<b>MBG</b>			
MPC1	10.49	47.68	47.68
MPC2	6.02	27.36	75.04
MPC3	1.74	7.91	82.95
<b>CV</b>			
CPC1	14.13	64.24	64.24
CPC2	3.61	16.40	80.64
CPC3	1.06	4.84	85.48
<b>J</b>			
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

<sup>1</sup>HPC, 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 <sup>1</sup>	BH1 <sup>2</sup>				BH2 <sup>2</sup>				MBG <sup>2</sup>			CV <sup>2</sup>			J <sup>2</sup>			
	HPC1	HPC2	HPC3	HPC4	BPC1	BPC2	BPC3	BPC4	MPC1	MPC2	MPC3	CPC1	CPC2	CPC3	JPC1	JPC2	JPC3	JPC4
C14:0	<b>0.957</b>	-0.152	-0.150	0.036	<b>0.916</b>	-0.294	-0.011	0.168	<b>0.940</b>	-0.233	0.134	<b>0.839</b>	<b>-0.510</b>	-0.042	<b>0.987</b>	0.059	0.029	-0.017
C15:0	0.174	-0.395	<b>0.647</b>	0.432	<b>0.831</b>	0.012	-0.220	-0.129	<b>0.867</b>	-0.008	0.123	<b>0.852</b>	-0.336	0.127	<b>0.876</b>	0.071	0.078	-0.267
C16:0	<b>0.983</b>	-0.122	-0.003	-0.015	<b>0.971</b>	-0.018	0.202	-0.026	<b>0.981</b>	-0.093	-0.077	<b>0.930</b>	-0.321	-0.143	<b>0.989</b>	0.118	0.010	0.006
C17:0	<b>0.933</b>	-0.142	-0.129	0.088	<b>0.916</b>	0.140	0.134	-0.099	<b>0.929</b>	0.067	0.001	<b>0.970</b>	-0.117	0.062	0.403	0.688	0.108	-0.261
C18:0	<b>0.879</b>	0.413	0.064	0.059	<b>0.769</b>	0.362	0.410	0.011	<b>0.795</b>	0.500	-0.177	<b>0.970</b>	0.152	-0.073	<b>0.868</b>	0.348	0.083	0.097
C20:0	0.673	0.633	0.081	-0.126	0.489	<b>0.720</b>	0.356	-0.231	0.431	0.639	<b>-0.548</b>	<b>0.868</b>	0.426	-0.117	0.239	<b>0.808</b>	-0.010	-0.322
C22:0	0.182	<b>0.865</b>	0.128	-0.064	0.091	<b>0.805</b>	-0.496	-0.120	-0.230	<b>0.815</b>	-0.148	<b>0.725</b>	0.470	-0.201	-0.147	<b>0.752</b>	-0.096	-0.110
C24:0	0.211	<b>0.892</b>	0.029	-0.213	-0.278	0.692	0.505	-0.064	-0.052	0.915	-0.138	0.578	<b>0.572</b>	-0.245	-0.531	<b>0.742</b>	-0.084	-0.195
C14:1	-	-	-	-	0.527	-0.514	-0.170	0.540	<b>0.811</b>	-0.140	0.435	<b>0.720</b>	-0.430	0.125	<b>0.904</b>	0.013	0.002	0.279
C16:1	<b>0.862</b>	-0.357	-0.149	-0.045	<b>0.932</b>	-0.213	0.096	-0.083	<b>0.925</b>	-0.181	0.198	0.633	<b>-0.689</b>	-0.039	<b>0.974</b>	0.089	-0.027	0.116
C18:1(n-9)c	<b>0.931</b>	-0.312	0.048	-0.128	<b>0.981</b>	-0.091	0.133	-0.046	<b>0.931</b>	-0.175	-0.191	<b>0.805</b>	<b>-0.544</b>	-0.147	<b>0.983</b>	-0.047	-0.037	-0.003
C20:1	<b>0.889</b>	-0.279	0.220	-0.171	<b>0.938</b>	0.039	0.169	-0.122	<b>0.927</b>	-0.083	-0.310	<b>0.857</b>	-0.269	-0.101	<b>0.932</b>	-0.051	-0.093	-0.011
C22:1(n-9)	-0.096	<b>0.917</b>	0.083	0.229	0.278	0.506	<b>-0.733</b>	0.218	-0.015	<b>0.705</b>	<b>0.576</b>	<b>0.842</b>	0.460	-0.029	-0.503	0.677	0.287	0.171
C24:1	0.319	<b>0.830</b>	0.092	-0.240	-0.346	<b>0.885</b>	0.095	-0.043	-0.141	<b>0.882</b>	-0.246	<b>0.904</b>	0.275	0.131	-0.344	<b>0.739</b>	-0.429	-0.171
C18:2(n-6)c	<b>0.982</b>	-0.080	0.015	0.024	<b>0.963</b>	-0.024	0.196	0.081	<b>0.979</b>	-0.117	-0.077	<b>0.934</b>	-0.262	-0.136	<b>0.982</b>	0.056	0.068	0.043
C18:3(n-6)	0.697	-0.215	-0.148	0.453	<b>0.797</b>	0.019	-0.253	0.100	<b>0.742</b>	-0.076	0.100	0.504	0.044	<b>0.711</b>	<b>0.751</b>	-0.009	0.296	-0.214
C20:2(n-6)	0.531	0.678	-0.020	0.318	0.443	<b>0.747</b>	0.153	0.181	0.078	0.660	0.192	<b>0.788</b>	0.393	0.193	0.272	0.618	0.353	0.380
C20:3(n-6)	0.177	<b>0.810</b>	0.075	0.214	-0.082	0.688	0.363	0.301	0.378	0.662	0.330	<b>0.730</b>	0.332	-0.183	0.156	0.599	-0.353	0.472
C20:4(n-6)	0.224	<b>0.718</b>	-0.350	-0.335	-0.341	-0.209	<b>0.892</b>	0.012	0.415	<b>0.715</b>	-0.399	<b>0.831</b>	0.361	0.148	0.261	0.182	-0.819	0.060
C18:3(n-3)	<b>0.927</b>	-0.347	-0.078	-0.034	<b>0.967</b>	-0.157	0.070	0.069	<b>0.934</b>	-0.262	-0.031	<b>0.803</b>	<b>-0.536</b>	0.024	<b>0.984</b>	0.106	0.032	-0.023
C20:5(n-3)	-0.218	0.435	<b>-0.581</b>	<b>0.531</b>	0.285	0.681	-0.524	0.153	0.438	0.638	0.304	0.606	0.328	0.423	-0.333	0.297	0.162	<b>0.786</b>
C22:6(n-3)	-0.026	<b>0.704</b>	0.382	0.114	-0.352	0.155	0.345	<b>0.783</b>	0.013	<b>0.740</b>	0.401	<b>0.729</b>	0.436	-0.198	-0.512	0.541	0.444	-0.214

<sup>1</sup>The loading displayed in **boldface** were variables contributed greatly to the principal components.<sup>2</sup>HPC, 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.



**Figure 1.** Effects of breeds/strains on the expression levels of lipid metabolism-related genes in duck breast muscle. <sup>a-d</sup> 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♂ × (BGF2♂ × GF2♀)♀. Genes: acetyl-CoA carboxylase, *ACACA*; carnitine palmitoyltransferase 1A, *CPT1A*; elongation of very long chain fatty acid 2, *ELOVL2*; 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*.

### Correlation of PC with Fatty Acids Composition Parameters and Genes Expression Levels

The scores of PC was 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,  $\Sigma\text{MUFA}/\Sigma\text{SFA}$ ,  $\Sigma\text{PUFA}/\Sigma\text{SFA}$ , and  $\Sigma n-6/\Sigma n-3$  were positively correlated with PC1 in five duck breeds/strains. Further analysis showed that the  $\Sigma\text{MUFA}/\Sigma\text{SFA}$ ,  $\Sigma\text{PUFA}/\Sigma\text{SFA}$ , and  $\Sigma n-6/\Sigma n-3$  ratios were negatively correlated with PC2 and PC4 in BH1 and BH2, with PC2 and PC3 in MBG and CV, but with PC3 in J, while they were positively correlated with PC3 in BH1 and BH2. As for PC and genes expression levels, HPC1, HPC3, and JPC1 were positively correlated with the mRNA levels of *ACACA* ( $P < 0.05$ ), whereas MPC2 was positively correlated with those of *FABP3* ( $P < 0.05$ ). BPC2 was negatively correlated with the mRNA levels of *ACACA* ( $P < 0.05$ ), and BPC4 was negatively correlated with those of *ELOVL5* ( $P < 0.05$ ).

## 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  $\Sigma\text{MUFA}$  was the highest in the 5 duck breeds/strains, while previous studies in other duck breeds showed that  $\Sigma\text{SFA}$  was the highest (Chen et al., 2016; Franco et al., 2012), 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 (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  $\Sigma\text{PUFA}/\Sigma\text{SFA}$  and  $\Sigma\text{MUFA}/\Sigma\text{SFA}$  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),

**Table 6.** Factor score coefficients from PCA of the five different duck breeds/strains.

Items	BH1 <sup>1</sup>				BH2 <sup>1</sup>				MBG <sup>1</sup>			CV <sup>1</sup>			J <sup>1</sup>			
	HPC1	HPC2	HPC3	HPC4	BPC1	BPC2	BPC3	BPC4	MPC1	MPC2	MPC3	CPC1	CPC2	CPC3	JPC1	JPC2	JPC3	JPC4
C14:0	0.103	-0.023	-0.124	0.031	0.088	-0.061	-0.004	0.132	0.090	-0.039	0.077	0.059	-0.141	-0.039	0.090	0.013	0.019	-0.011
C15:0	0.019	-0.059	0.533	0.365	0.080	0.002	-0.074	-0.101	0.083	-0.001	0.071	0.060	-0.093	0.119	0.080	0.016	0.050	-0.174
C16:0	0.105	-0.018	-0.002	-0.013	0.093	-0.004	0.068	-0.020	0.094	-0.015	-0.044	0.066	-0.089	-0.134	0.090	0.026	0.006	0.004
C17:0	0.100	-0.021	-0.106	0.075	0.088	0.029	0.045	-0.078	0.089	0.011	0.000	0.069	-0.032	0.058	0.037	0.150	0.069	-0.170
C18:0	0.094	0.062	0.053	0.050	0.074	0.075	0.137	0.009	0.076	0.083	-0.102	0.069	0.042	-0.068	0.079	0.076	0.053	0.063
C20:0	0.072	0.095	0.067	-0.106	0.047	0.149	0.119	-0.181	0.041	0.106	-0.315	0.061	0.118	-0.110	0.022	0.177	-0.006	-0.210
C22:0	0.020	0.130	0.105	-0.054	0.009	0.167	-0.166	-0.094	-0.022	0.135	-0.085	0.051	0.130	-0.189	-0.013	0.165	-0.062	-0.071
C24:0	0.023	0.134	0.024	-0.180	-0.027	0.143	0.169	-0.050	-0.005	0.152	-0.079	0.041	0.159	-0.230	-0.048	0.162	-0.054	-0.127
C14:1	-	-	-	-	0.051	-0.106	-0.057	0.424	0.077	-0.023	0.250	0.051	-0.119	0.117	0.082	0.003	0.001	0.181
C16:1	0.093	-0.054	-0.122	-0.038	0.089	-0.044	0.032	-0.065	0.088	-0.030	0.114	0.045	-0.191	-0.037	0.088	0.019	-0.018	0.076
C18:1(n-9)c	0.100	-0.047	0.040	-0.108	0.094	-0.019	0.044	-0.036	0.089	-0.029	-0.110	0.057	-0.151	-0.138	0.089	-0.010	-0.024	-0.002
C20:1	0.095	-0.042	0.181	-0.145	0.090	0.008	0.057	-0.096	0.088	-0.014	-0.178	0.061	-0.075	-0.095	0.085	-0.011	-0.060	-0.007
C22:1(n-9)	-0.010	0.138	0.069	0.193	0.027	0.105	-0.246	0.171	-0.001	0.117	0.331	0.060	0.127	-0.028	-0.046	0.148	0.185	0.111
C24:1	0.034	0.125	0.076	-0.203	-0.033	0.183	0.032	-0.034	-0.013	0.147	-0.141	0.064	0.076	0.123	-0.031	0.162	-0.276	-0.111
C18:2(n-6)c	0.105	-0.012	0.013	0.020	0.092	-0.005	0.066	0.064	0.093	-0.019	-0.044	0.066	-0.073	-0.127	0.089	0.012	0.044	0.028
C18:3(n-6)	0.075	-0.032	-0.122	0.383	0.076	0.004	-0.085	0.079	0.071	-0.013	0.057	0.036	0.012	0.668	0.068	-0.002	0.190	-0.139
C20:2(n-6)	0.057	0.102	-0.016	0.268	0.042	0.155	0.051	0.142	0.007	0.110	0.111	0.056	0.109	0.182	0.025	0.135	0.227	0.247
C20:3(n-6)	0.019	0.122	0.062	0.181	-0.008	0.143	0.122	0.236	0.036	0.110	0.190	0.052	0.092	-0.172	0.014	0.131	-0.227	0.308
C20:4(n-6)	0.024	0.108	-0.288	-0.283	-0.033	-0.043	0.299	0.010	0.040	0.119	-0.229	0.059	0.100	0.139	0.024	0.040	-0.526	0.039
C18:3(n-3)	0.099	-0.052	-0.064	-0.028	0.093	-0.033	0.024	0.054	0.089	-0.043	-0.018	0.057	-0.149	0.023	0.089	0.023	0.020	-0.015
C20:5(n-3)	-0.023	0.065	-0.478	0.449	0.027	0.141	-0.175	0.120	0.042	0.106	0.175	0.043	0.091	0.397	-0.030	0.065	0.104	0.512
C22:6(n-3)	-0.003	0.106	0.315	0.097	-0.034	0.032	0.116	0.615	0.001	0.123	0.230	0.052	0.121	-0.186	-0.047	0.118	0.285	-0.139

<sup>1</sup>HPC, 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's correlation analysis of principal composition, fatty acids composition, and genes expression

Items	BH1 <sup>1</sup>				BH2 <sup>1</sup>				MBG <sup>1</sup>				CV <sup>1</sup>				J <sup>1</sup>			
	HPC1	HPC2	HPC3	HPC4	BPC1	BPC2	BPC3	BPC4	MPC1	MPC2	MPC3	CPC1	CPC2	CPC3	JPC1	JPC2	JPC3	JPC4		
Genes																				
ACACA	0.828*	-0.653	0.870*	-0.669	0.541	-0.925*	0.607	-0.753	-0.250	-0.303	-0.176	0.513	-0.201	-0.407	0.896*	-0.052	-0.420	0.400		
SCD1	0.167	-0.450	0.008	-0.428	0.213	-0.766	0.286	-0.578	0.244	-0.724	-0.451	0.823	-0.746	-0.824	-0.256	0.861	0.856	0.551		
FADS1	0.639	-0.750	0.547	-0.743	0.031	-0.463	0.102	-0.552	-0.347	0.179	0.125	0.261	-0.189	-0.263	0.414	0.382	0.363	0.428		
FADS2	0.069	0.138	0.041	-0.134	0.244	-0.449	0.311	-0.740	-0.390	0.388	0.268	0.250	-0.084	-0.231	0.710	-0.326	-0.557	0.088		
ELOVL2	0.360	-0.205	0.444	-0.231	-0.570	-0.367	-0.510	0.085	-0.783	0.849	0.810	-0.798	0.676	0.756	-0.413	0.391	0.741	0.026		
ELOVL5	0.192	-0.191	0.197	-0.193	0.832	-0.722	0.875	-0.889*	-0.300	0.823	0.682	0.440	-0.349	-0.443	-0.459	0.650	0.848	0.259		
CPT1A	-0.192	0.112	-0.246	0.130	0.717	-0.461	0.751	-0.850	0.042	0.236	-0.038	0.733	-0.589	-0.711	0.046	-0.330	-0.200	-0.264		
FATB1	-0.133	0.354	-0.015	0.336	0.773	0.518	0.783	-0.594	-0.120	0.064	-0.049	-0.388	0.266	0.341	-0.093	-0.077	0.207	-0.186		
FABP3	0.171	-0.279	0.114	-0.281	0.967*	-0.434	0.962*	-0.656	-0.449	0.889*	0.759	0.413	-0.159	-0.314	-0.295	0.619	0.777	0.289		
SREBP1	0.420	-0.510	0.365	-0.513	0.794	-0.174	0.779	-0.540	0.759	-0.584	-0.829	-0.004	-0.075	-0.051	0.037	0.084	-0.192	0.178		
Fatty acid composition																				
ΣMUFA/ΣSFA	0.824**	-0.973**	0.699**	-0.964**	0.806**	-0.380	0.347	-0.468*	0.821**	-0.859**	-0.835**	0.376	-0.711**	-0.479*	0.941**	0.075	-0.577**	0.631**		
ΣPUFA/ΣSFA	0.703**	-0.784**	0.583**	-0.754**	0.211	-0.737**	0.666**	-0.354	0.495*	-0.714**	-0.527**	0.107	-0.295	-0.161	0.870**	0.148	-0.375	0.654**		
Σn-6/Σn-3	0.809**	-0.771**	0.744**	-0.791**	0.305	-0.736**	0.765**	-0.693	0.572**	-0.704**	-0.665**	0.219	-0.383	-0.278	0.923**	0.040	-0.588**	0.626		

\* and \*\* indicated the significance of correlations.

<sup>1</sup>HPC, principal components of BH1; BPC, principal components of BH2; MPC, principal components of MBG; CPC, principal components of CV; JPC, principal components of J. Abbreviations: MUFA: mono-unsaturated fatty acid; n-3: omega 3; n-6: omega 6; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acid.

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. Further 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  $\Sigma$ MUFA/ $\Sigma$ SFA,  $\Sigma$ PUFA/ $\Sigma$ SFA, and  $\Sigma n-6/\Sigma n-3$  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 BH2 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 *SCD1*, *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  $\Sigma$ PUFA/ $\Sigma$ SFA and  $\Sigma$ MUFA/ $\Sigma$ SFA but lower  $\Sigma n-6/\Sigma n-3$  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.

## FUNDING

This research was supported by China Agriculture Research System of MOF and MARA (CARS-42-4), School Cooperation Project of Ya' an (21SXHZ0028), and Key Technology Support Program of Sichuan Province (2021YFYZ0014) for the financial support.

## DISCLOSURES

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

## REFERENCES

- Arshad, M. S., M. Sohaib, R. S. Ahmad, M. T. Nadeem, A. Imran, M. U. Arshad, J. H. Kwon, and Z. Amjad. 2018. Ruminant meat flavor influenced by different factors with special reference to fatty acids. *Lipids. Health Dis.* 17:223.
- Castro, L. F., D. R. Tocher, and O. Monroig. 2016. Long-chain polyunsaturated fatty acid biosynthesis in chordates: insights into the evolution of Fads and Elovl gene repertoire. *Prog. Lipid. Res.* 62:25–40.
- Chen, Y., Y. Qiao, Y. Xiao, H. Chen, L. Zhao, M. Huang, and G. Zhou. 2016. Differences in physicochemical and nutritional properties of breast and thigh meat from crossbred chickens, commercial broilers, and spent hens. *Asian-Austr. J. Anim. Sci.* 29:855–864.
- Cömert, M., Y. Şayan, F. Kırkpınar, H. Bayraktar Ö., and S. Mert. 2016. Comparison of carcass characteristics, meat quality, and blood parameters of slow and fast grown female broiler chickens raised in organic or conventional production system. *Asian-Austr. J. Anim. Sci.* 29:987–997.
- Cui, L. L., J. F. Wang, K. Z. Xie, A. H. Li, T. Y. Geng, L. R. Sun, J. Y. Liu, M. Zhao, G. X. Zhang, G. J. Dai, and J. Y. Wang. 2015. Analysis of meat flavor compounds in pedigree and two-strain Yangzhou geese. *Poult Sci* 94:2266–2271.
- Cui, X. Y., Z. Y. Gou, K. F. M. Abouelezz, L. Li, X. J. Lin, Q. L. Fan, Y. B. Wang, Z. G. Cheng, F. Y. Ding, and S. Q. Jiang. 2020. Alterations of the fatty acid composition and lipid metabolome of breast muscle in chickens exposed to dietary mixed edible oils. *Animal* 14:1322–1332.
- de Bus, I., R. Witkamp, H. Zuilhof, B. Albada, and M. Balvers. 2019. The role of n-3 PUFA-derived fatty acid derivatives and their oxygenated metabolites in the modulation of inflammation. *Prostaglandins Other Lipid Mediat.* 144:106351.
- Ding, S. R., G. S. Li, S. R. Chen, F. Zhu, J. P. Hao, F. X. Yang, and Z. C. Hou. 2021. Comparison of carcass and meat quality traits between lean and fat Pekin ducks. *Anim. Biosci.* 34:1193–1201.
- Ding, S. T., A. P. Schinckel, T. E. Weber, and H. J. Mersmann. 2000. Expression of porcine transcription factors and genes related to fatty acid metabolism in different tissues and genetic populations. *J. Anim. Sci.* 78:2127–2134.
- Fan, W., W. Liu, H. Liu, Q. Meng, Y. Xu, Y. Guo, B. Wang, Z. Zhou, and S. Hou. 2020. Dynamic accumulation of fatty acids in duck (*Anas platyrhynchos*) breast muscle and its correlations with gene expression. *BMC Genom.* 21:58.
- Frampton, J., K. G. Murphy, G. Frost, and E. S. Chambers. 2020. Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. *Nat. Metab.* 2:840–848.
- Franco, D., D. Rois, J. A. Vázquez, and J. M. Lorenzo. 2012. Comparison of growth performance, carcass components, and meat quality between Mos rooster (Galician indigenous breed) and Sasso T-44 line slaughtered at 10 months. *Poult. Sci.* 91:1227–1239.
- Gaur, U., M. S. Tandia, B. Mishra, S. T. Bharani Kumar, R. K. Vijh, and A. Chaudhury. 2018. Mitochondrial D-loop analysis for uncovering the population structure and genetic diversity among the indigenous duck (*Anas platyrhynchos*) populations of India. *Mitochondrial DNA A DNA Mapp. Seq. Anal.* 29:212–219.
- Gerbens, F., A. J. van Erp, F. L. Harders, F. J. Verburg, T. H. Meuwissen, J. H. Veerkamp, and M. F. te Pas. 1999. Effect of genetic variants of the heart fatty acid-binding protein gene on intramuscular fat and performance traits in pigs. *J. Anim. Sci.* 77:846–852.
- Glaser, C., J. Heinrich, and B. Koletzko. 2010. Role of FADS1 and FADS2 polymorphisms in polyunsaturated fatty acid metabolism. *Metabolism* 59:993–999.
- Gou, Z. Y., X. Y. Cui, L. Li, Q. L. Fan, X. J. Lin, Y. B. Wang, Z. Y. Jiang, and S. Q. Jiang. 2020. Effects of dietary incorporation of linseed oil with soybean isoflavone on fatty acid profiles and lipid metabolism-related gene expression in breast muscle of chickens. *Animal* 14:2414–2422.
- Hoa, V. B., S. H. Cho, P. N. Seong, S. M. Kang, Y. S. Kim, S. S. Moon, Y. M. Choi, J. H. Kim, and K. H. Seol. 2020. Quality characteristics, fatty acid profiles, flavor compounds and eating quality of cull sow meat in comparison with commercial pork. *Asian-Austr. J. Anim. Sci.* 33:640–650.
- Huang, J., R. Zhu, and D. Shi. 2021. The role of FATP1 in lipid accumulation: a review. *Mol. Cell Biochem.* 476:1897–1903.
- Huang, Y., L. Zhou, J. Zhang, X. Liu, Y. Zhang, L. Cai, W. Zhang, L. Cui, J. Yang, J. Ji, S. Xiao, H. Ai, C. Chen, J. Ma, B. Yang, and L. Huang. 2020. A large-scale comparison of meat quality and intramuscular fatty acid composition among three Chinese indigenous pig breeds. *Meat Sci.* 168:108182.
- Janiszewski, P., D. Murawska, V. Hanzal, M. Gesek, D. Michalik, and M. Zawacka. 2018. Carcass characteristics, meat quality, and fatty

- acid composition of wild-living mallards (*Anas platyrhynchos* L.). *Poult. Sci.* 97:709–715.
- Jiang, T., C. J. Mueller, J. R. Busboom, M. L. Nelson, J. O'Fallon, and G. Tschida. 2013. Fatty acid composition of adipose tissue and muscle from Jersey steers was affected by finishing diet and tissue location. *Meat Sci.* 93:153–161.
- Kouba, M., F. Benatmane, J. E. Blochet, and J. Mourot. 2008. Effect of a linseed diet on lipid oxidation, fatty acid composition of muscle, perirenal fat, and raw and cooked rabbit meat. *Meat Sci.* 80:829–834.
- Liu, Y., D. Wan, X. Zhou, Z. Ruan, T. Zhang, X. Wu, and Y. Yin. 2019. Effects of dynamic feeding low- and high-methionine diets on the variation of glucose and lipid metabolism-related genes in the liver of laying hens. *Poult. Sci.* 98:2231–2240.
- Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods* 25:402–408.
- Nakamura, M. T., B. E. Yudell, and J. J. Loor. 2014. Regulation of energy metabolism by long-chain fatty acids. *Prog. Lipid. Res.* 53:124–144.
- Onk, K., H. Yalcintan, M. Sari, S. Adiguzel Isik, A. Yakan, and B. Ekiz. 2019. Effects of genotype and sex on technological properties and fatty acid composition of duck meat. *Poult. Sci.* 98:491–499.
- Peng, Y. D., H. Y. Xu, F. Ye, X. Lan, X. Peng, A. Rustempašić, H. D. Yin, X. L. Zhao, Y. P. Liu, Q. Zhu, and Y. Wang. 2015. Effects of sex and age on chicken TBC1D1 gene mRNA expression. *Genet. Mol. Res.* 14:7704–7714.
- Rottiers, V., S. H. Najafi-Shoushtari, F. Kristo, S. Gurumurthy, L. Zhong, Y. Li, D. E. Cohen, R. E. Gerszten, N. Bardeesy, R. Mostoslavsky, and A. M. Näär. 2011. MicroRNAs in metabolism and metabolic diseases. *Cold Spring Harb. Symp. Quant. Biol.* 76:225–233.
- Rymer, C., and D. I. Givens. 2005. n-3 fatty acid enrichment of edible tissue of poultry: a review. *Lipids* 40:121–130.
- Temple, N. J. 2018. Fat, sugar, whole grains and heart disease: 50 years of confusion. *Nutrients* 10.
- Tomaz̃in, U., N. Batorek-Lukač, M. Škrlep, M. Prevolnik-Povše, and M. Čandek-Potokar. 2019. Meat and fat quality of Krškopolje pigs reared in conventional and organic production systems. *Animal* 13:1103–1110.
- Tu, T., W. Wu, X. Tang, Q. Ge, and J. Zhan. 2021. Screening out important substances for distinguishing Chinese indigenous pork and hybrid pork and identifying different pork muscles by analyzing the fatty acid and nucleotide contents. *Food Chem.* 350:129219.
- Wang, G., W. K. Kim, M. A. Cline, and E. R. Gilbert. 2017. Factors affecting adipose tissue development in chickens: a review. *Poult. Sci.* 96:3687–3699.
- Wotoszyn, J., G. Haraf, A. Okruszek, M. Wereńska, Z. Goluch, and M. Teleszko. 2020. Fatty acid profiles and health lipid indices in the breast muscles of local Polish goose varieties. *Poult. Sci.* 99:1216–1224.
- Xie, Z., J. Zhang, S. Ma, X. Huang, and Y. Huang. 2017. Effect of Chinese herbal medicine treatment on plasma lipid profile and hepatic lipid metabolism in Hetian broiler. *Poult. Sci.* 96:1918–1924.
- Xu, H., J. Luo, H. Tian, J. Li, X. Zhang, Z. Chen, M. Li, and J. J. Loor. 2018. Rapid communication: lipid metabolic gene expression and triacylglycerol accumulation in goat mammary epithelial cells are decreased by inhibition of SREBP-1. *J. Anim. Sci.* 96:2399–2407.
- Yalcintan, H., B. Ekiz, and M. Ozcan. 2018. Comparison of meat quality characteristics and fatty acid composition of finished goat kids from indigenous and dairy breeds. *Trop. Anim. Health Prod.* 50:1261–1269, doi:10.1007/s11250-018-1553-3.
- Yu, K., G. Shu, F. Yuan, X. Zhu, P. Gao, S. Wang, L. Wang, Q. Xi, S. Zhang, Y. Zhang, Y. Li, T. Wu, L. Yuan, and Q. Jiang. 2013. Fatty acid and transcriptome profiling of longissimus dorsi muscles between pig breeds differing in meat quality. *Int. J. Biol. Sci.* 9:108–118.
- Zhang, Q., H. Shi, W. Liu, Y. Wang, Q. Wang, and H. Li. 2013. Differential expression of L-FABP and L-BABP between fat and lean chickens. *Genet. Mol. Res.* 12:4192–4206.
- Zhang, S., T. J. Knight, K. J. Stalder, R. N. Goodwin, S. M. Lonergan, and D. C. Beitz. 2007. Effects of breed, sex, and halothane genotype on fatty acid composition of pork longissimus muscle. *J. Anim. Sci.* 85:583–591.
- Zhang, Y., J. Zhang, H. Gong, L. Cui, W. Zhang, J. Ma, C. Chen, H. Ai, S. Xiao, L. Huang, and B. Yang. 2019. Genetic correlation of fatty acid composition with growth, carcass, fat deposition and meat quality traits based on GWAS data in six pig populations. *Meat Sci.* 150:47–55.
- Zhu Cai-Ye, W. G.-H., W. Li, D. Wang, M.-M. Zheng, Z.-Y. Liu, Y.-N. Zhang, and B.-C. Li. 2013. Cloning of Xuhuai Goat SCD1 gene, subcellular localization and the preparation of transgenic mice. *Scientia Agricultura Sinica* 46:3695–3703.