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Original Research Article

Integrated metabolomics and microbiome analysis reveal blended oil diet improves meat quality of broiler chickens by modulating flavor and gut microbiota

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ABSTRACT

This study was to evaluate the effects of different dietary oils in chicken diets on meat quality, lipid metabolites, the composition of volatile compounds, and gut microbiota. Nine hundred female 817 crossbred broilers at one day old with an average body weight of 43.56 ± 0.03 g were randomly divided into five treatments, each consisting of 6 replicates of 30 birds. The control group received soybean oil (SO); other groups received diets supplemented with rice bran oil (RO), lard (LO), poultry fat (PO), and blended oil (BO), respectively. All diets were formulated as isoenergic and isonitrogenous. Compared with SO, RO decreased ADG and 42 d BW ($P < 0.05$). Compared with the RO, BO increased ADG and 42 d BW and decreased FCR ($P < 0.05$). Compared with SO, BO increased 24 h redness (a*) value and reduced the malondialdehyde concentration ($P < 0.05$), and further improved drip loss of breast muscle ($P > 0.05$). The proportions of C18:0 and saturated fatty acid were the highest in LO, and the proportions of C16:1, C18:1, and monounsaturated fatty acids were the highest in BO. The content of C18:2, C18:3, and polyunsaturated fatty acids were the highest in SO. The contents of glyceryl triglycerides and total esters in BO were significantly higher than those in the SO and LO group ($P < 0.05$). There was a substantial increment in the relative abundance of peroxisome proliferator activated receptor alpha (*PPARα*), acyl-CoA oxidase 1 (*ACOX1*), and carnitine palmitoyl-transferase 1 (*CPT1A*) transcripts in breast of chickens fed BO ($P < 0.05$). Further, dietary BO increased the relative cecal abundance of Firmicutes phylum, *Ruminococcus_torques* and *Christensenellaceae_R-7* genera, and decreased that of Campylobacterota, Proteobacteria, and *Phascolarctobacterium* ($P < 0.05$). Genera *g_Lactobacillus* and *Christensenellaceae_R-7* may mainly be involved in the formation of volatile flavor compounds in breast muscle. In conclusion, dietary BO improved the flavor of chickens by increasing the concentration of triglycerides and volatile

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flavor compounds, improving gut microbiota structure, and suppressing lipid oxidation. The potential positive effects of BO may be associated with the regulation of lipid metabolism.

1. Introduction

Lipids are high-energy density ingredients in feedstuffs, which make them desirable in feed mills as they reduce dust and aid pelleting; they are also important in providing specific critical nutrients (Oketch et al., 2023). Lipids such as soybean oil (SO), rice bran oil (RO), pork-derived lard (LO), and poultry fat are commonly used as raw materials for their high caloric density as well as being the source of multiple essential fatty acids for growth (Saleh et al., 2021; Song et al., 2022). To date, it has not been well understood how dietary fats and oils impact meat quality and flavor, as well as the nutritional balance of fatty acids.

Palm oil is a relatively stable substance that is rich in medium chain fatty acids (MCFA) of C6:0 and C8:0. Additionally, it contains antioxidants, including vitamin E, carotenoids, polyphenols, flavonoids, and coenzyme Q10 (Absalome et al., 2020). Coconut oil is also rich in MCFA, in particular lauric acid (Deen et al., 2021), which has been implicated in improving performance, intestinal absorption, immunity, and altering lipid metabolism, and the intestinal microbiota in broiler chickens (Wu et al., 2021). In poultry diets, phospholipids are an ingredient rich in unsaturated fatty acids and are able to suppress body fat deposition and enhance liver functions including lipid metabolism (Yang et al., 2017). Although fast-growing broilers have potential for meat production, the meat tenderness and flavor decline as the maturation period is accelerated. Compared with individual oils, blended or mixed oils are thought to be ideal due to their optimal fatty acid compositions, which potentially help to promote broiler growth and improve their meat quality and flavor. In this study we used a novel commercially available blended oil (BO) lipid product which consists of soybean phospholipids, palm oil and coconut oil.

The color, tenderness, and flavor of chicken meat greatly influence consumer choice and preference. Intramuscular fat (IMF) content and fatty acid composition have a significant influence on meat quality, especially meat flavor. Volatile organic compounds (VOC) are essential due to their effect on the flavor of chicken meat. The type and abundance of VOC are closely related to lipid metabolism in chickens and thus chicken meat fatty acid compositions (Dinh et al., 2021). The intestinal microbiota is a complex ecosystem and some communities are thought to affect meat quality and flavor of chickens (Sun et al., 2018). Until now, no information has been available regarding the impact of the most common fat or oil types used commercially in poultry diets, or novel BO on intestinal microbiota or VOC in chicken meat. To address this gap in knowledge, in the present study, fast-growing broilers were fed different dietary fats and oils to investigate the effects of different lipids on meat quality and gut microbiota. Lipidomics and flavoromics, based on liquid chromatography–mass spectrometry (LC–MS) and gas chromatography with mass spectrometry (GC–MS), were used to further explore the dietary fat-induced changes in lipid composition and volatile flavor compounds, which may help identify the mechanisms of the observed effect of oils on flavor formation.

2. Materials and methods

2.1. Animal ethics statement

The research protocol was approved by the Animal Ethics Committee of the Institute of Animal Science, Guangdong Academy of Agriculture Sciences (No. 2022017).

2.2. Animals, experimental design and diets

Nine hundred female 817 Crossbred broilers at one day old, a commercial hybrid strain, were purchased from Guangdong Wenshi Southern Poultry Breeding Co., Ltd (Yunfu, Guangdong, China). The birds were randomly divided to five groups with 6 replicates each and 30 birds per replicate. Birds were raised for 50 days from 1 to 50 d old. All birds were kept in a floor pen (length 3.5 m × width 1.5 m) with a standard environment, and had free access to water and feed. Lighting was provided by incandescent bulbs with a lighting program of 23 h (23L:1D) of light and 1 h of darkness from 0 to 7 d of age, followed by 18 h (18L:6D) of light for the remainder of the trial. The temperature of the room was maintained at 32 to 34 °C for the first 3 d and then reduced by 2 to 3 °C per week to a final temperature at 26 °C from d 1 to 21, and the temperature was the same for older chickens.

Diets based on corn–wheat–soybean meal were formulated to meet nutrient requirements for chickens according to the requirements of the yellow-feathered broiler recommended by the Ministry of Agriculture of China (NY/T 3645-2020) with adjustment according to the age (1 to 21 d, 22 to 42 d, and 43 to 50 d). The composition and nutrient levels are shown in supplementary material (Tables S1 to S3). The five experimental diets contained SO, RO, LO, poultry-derived oil (PO), or a BO (FeiYou, Youbaite Biotechnology Co., Ltd, Guangzhou, China) which was a mixture of 50% soybean phospholipids, 35% palm oil, and 15% coconut oil, respectively. All diets were isoenergic and isonitrogenous at each stage. To ensure freshness, the SO and RO were purchased from a local grain and oil market and LO, and PO were purchased from Guangdong Wenshi Feed Co., Ltd. (Yunfu, Guangdong, China) with known tested acid and peroxide values. The oil and fat were packaged and stored at 0 °C until use. Samples of the mixed feeds for these diets and periods were collected and stored at −80 °C for later analysis of chemical composition.

Nutrient levels in the experimental diet were analyzed by the following methods or calculated according to the requirement of the Yellow-feathered broilers recommended by the Ministry of Agriculture of China (NY/T 3645-2020). Briefly, the crude protein, methionine and cystine, other amino acids, crude fat, crude fiber, Ca, and total P were analyzed according to China National Standard (GB/T 6432-2018, GB/T 15399-2018, GB/T 18246-2019, GB/T 6433-2006, GB/T 6434-2022, GB/T 6436-2018, and GB/T 6437-2018). The fatty acid composition was analyzed as described previously (Folch et al., 1957) and is shown in Tables S4 to S6.

2.3. Growth performance

Chickens and remaining feed were weighed by pen at 1, 21, 42, and 50 d of age. The values for live weight, average daily feed intake, average daily gain, and feed conversion ratio were calculated. Mortality rates were recorded daily for the calculation of mortality.

2.4. Carcass traits and preparation of tissue samples

On 50 d old, two birds that had average body weight in each replicate were selected. These birds were fasted before being stunned and euthanized by exsanguination. The carcass was weighed both half-eviscerated and fully eviscerated, along with breast muscle and leg muscle weights which were measured to calculate their relative weights. The abdominal adipose tissue was weighed to calculate the abdominal fat rate, as described previously (Song et al., 2022). The breast muscle samples from the left side of the carcass were collected to measure pH, drip loss, meat color, and shear force. The breast muscle sample (about 200 g) from the left side of the carcass was frozen at −20 °C for later measurements of muscle fatty acid composition. In addition, samples of each right breast muscle (12 birds per group) were frozen in liquid nitrogen for the RNA extraction, and measurement of lipid metabolites and VOC. Cecal digesta samples were harvested and frozen at −80 °C for later analysis of gut microbiota.

2.5. Meat quality

Intramuscular pH was measured at 45 min and 24 h after slaughter by directly inserting a Testo 205 pH meter electrode (Testo AG, Lenzkirch, Germany) into three locations along the pectoralis major muscle which was stored in a refrigerator at 4 °C for 24 h. Meat color variables including lightness (L*), redness (a*), and yellowness (b*) were measured in triplicate at various locations at 45 min and 24 h after slaughter using a portable chromameter (CR-300, Minolta, Osaka, Japan). Drip loss was determined by measuring the amount of fluid lost after storing breast muscle samples in plastic bags at 4 °C for 24 h. First, the filter paper was used to blot samples, and then the samples were weighed again. Drip loss was expressed as percent weight change. Shear force was measured using a texture analyzer (Model LRX, Lloyd Instruments, Hampshire, UK) as stated previously (Knight et al., 2019).

2.6. Fatty acid composition analysis

The breast muscle specimens were evaluated for fatty acid composition using gas chromatography (Agilent, Santa Clara, CA, USA). The method of Folch et al. (1957) was followed to extract total lipid from breast muscle. The fatty acid methyl esters were separated by gas chromatography comprising an Agilent DB-1 fused silica capillary column (30 m × 250 μm × 0.25 μm). Identification of fatty acids was based on the retention time using a GC–MS QP2010-Plus (Shimadzu, Kyoto, Japan). The percentage of identified fatty acids was calculated based on the total fatty acids.

2.7. Untargeted lipidomic analysis

Untargeted lipidomic analysis of chicken breast meat samples were performed using UPLC-Q-Orbitrap MS/MS (Thermo Fisher Scientific, Waltham, MA, USA) as outlined by a previous study (Jia et al., 2022). The 50 mg of sample was homogenized in 500 μL chloroform and methanol mixture (2:1, v:v), to which 150 μL aqueous solution was added and vortexed for 2 min followed by incubation for 10 min at 4 °C. After that, samples were centrifuged at 3800 × *g* for 15 min at 4 °C. An equal volume of the lower chloroform layer was transferred to a 1.5-mL EP tube and dried under liquid nitrogen and stored at −80 °C. The pellet was mixed with internal standards, containing phosphatidylcholine (PC, 14:0/14:0), phosphatidylglycerol (PG, 14:0/14:0), and fatty acid (FA, 18:0) all at 2 μg/mL, in 200 μL of chloroform and methanol mixture (2:1, v:v) to facilitate further analysis. Pooled aliquots of such treated samples were used for quality control. Heating electrospray ionization (HESI) in positive and negative ion modes was performed on a Q-Exactive Orbitrap MS linked to a Thermo Fisher Ultimate 3000 UPLC system. A Cortecs C18 column (2.1 × 100 mm, 1.8 μm; Waters, Milford, MA, USA) with a binary solvent system was used to obtain lipid extracts. The separation process employed in this study involved the use of a 60% acetonitrile aqueous solution (solvent A) and isopropyl alcohol with 10% acetonitrile (solvent B). Both solvents were supplemented with 10 mmol/L ammonium acetate under the following conditions: in the first 1 min, a linear gradient was applied, increasing the concentration of solvent B from 30% to 38%. From 1 to 4 min, the concentration of solvent B was further increased from 38% to 56%. Subsequently, from 4 to 14 min, the concentration of solvent B was increased from 56% to 98%. From 14 to 15 min, the concentration of solvent B was increased from 98% to 100%. From 15 to 16 min, a brief isocratic step was then performed at 100% solvent B. From 16 to 16.1 min, the concentration of solvent B was decreased from 100% to 30%. Finally, from 16.1 to 20 min, the concentration of solvent B was maintained at 30%. The mobile phase flow rate was 0.3 mL/min and injection volume 5 μL.

The data was acquired with data-dependent acquisition using the MS/MS system. The mass spectrometer conditions were: a spray voltage of 3200 V in positive mode and 2800 V in negative mode; flow rate of auxiliary gas at 10 arbitrary units; temperature of capillary at 320 °C; a mass range (*m/z*) of 240 to 2000 (positive) and 200 to 2000 (negative); the complete scan resolution at 70,000 dots per inch (dpi) and the fragmentation spectral resolution at 17,500 dpi; duty cycle at 1.2 s. Lipid Search software (v4.2, Mitsui Knowledge Industry, Tokyo, Japan) was used to identify lipids that matched precursors and distinctive lipid fragments. The relative peak areas of the detected lipids were utilized to express the relative quantification.

2.8. Analysis of volatile compounds

Volatile flavor compounds of the chicken breast samples were extracted using headspace solid phase microextraction (HS-SPME) and analyzed by a gas chromatography/mass spectrometry (GC/MS) system (QP2020 Shimadzu, Kyoto, Japan) with a capillary column (InertCapWaX, 60 mm × 0.25 mm × 0.25 μm) as mentioned by Wen et al. (2019). The 5.00-g samples of chicken breast samples were added with 0.5 μg 2-methyl-3-heptanone (0.05 μg/μL in methanol) as the internal standard, and deposited in a PTFE-silicone-capped headspace vial. After 20 min at 55 °C, samples were extracted using 75 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Inc., Bellefonte, PA, USA) and exposed to the HS-SPME vial at 55 °C for 40 min. The extraction head was then thermally desorbed at 250 °C for 3 min. A GC/MS system (QP2020 Shimadzu, Kyoto, Japan) with an InertCapWaX capillary column (60 mm × 0.25 mm × 0.25 μm, Agilent) was used to analyze VOC. A temperature program started at 40 °C and was held for 2 min before the temperature was ramped up to 230 °C at a rate of 4 °C/min for 5 min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The ion source temperature was set at 280 °C. The mass spectrometer scanning range was 30 to 450 *m/z*. Volatile organic

compounds were qualitatively analyzed by comparing the deconvoluted mass spectra obtained from NIST 14 mass spectral library and/or calculated by the linear retention index (LRI) relative to a mixture of n-alkanes (C6 to C20) under the same analytical conditions. VOC were quantified on the basis of recovery of the internal standard.

2.9. Full-length 16S rRNA sequencing of gut microbiota

Total genomic DNA from each cecal digesta sample was extracted using the Mag-Bind Soil DNA Kit (Omega, Norcross, GA, USA). All DNA samples were evaluated for purity and concentration using a Nanodrop ND-2000 (Thermo Fisher Scientific, Waltham, MA, USA). The V1 to V9 region of the full-length bacterial 16S rRNA genes were amplified with the 27F/1492R primer. The polymerase chain reaction (PCR) amplicons were purified using the Qiagen Gel Extraction Kit (Qiagen, Santa Clarita, CA, USA) and sequenced using the PacBio Sequel IIe platform (Majorbio Bio-Pharm Technology Co., Ltd, Shanghai, China). The bioinformatics analysis was performed as elucidated by a previous study (Pandit et al., 2018).

2.10. Quantitative real-time PCR (RT-qPCR)

Total RNA was extracted from breast muscle using TRIZOL reagent (TaKaRa, Dalian, China), and cDNA was synthesized using the PrimeScript RT reagent kit with gDNA eraser (TaKaRa) following the manufacturer's instructions. RT-qPCR was performed on a CFX96 Real-time qPCR system (Applied Biosystems, Bio-Rad, Hercules, CA, USA) with SYBR select Master Mix (Applied Biosystems). Table S7 provides the information on lipid metabolism-related genes and the primers synthesized and purchased from Sangon Biotech (Shanghai) Co., Ltd. (China). Gene transcription was measured using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The internal control, β-actin, was employed to normalize the expression of the target genes.

2.11. Statistical analysis

Data were expressed as means ± standard error of the mean (SEM). General statistical analysis was conducted using ANOVA with the GLM procedures of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and Duncan's multiple comparison tests were used to determine the differences between groups. Statistical significance was defined as $P < 0.05$. Pearson's correlation was applied to analyze the co-occurrence relationship between lipid metabolites and gut microbiota as well as VOC, and features were considered significant at $P < 0.05$.

3. Results

3.1. Growth performance and carcass traits

As shown in Table 1, RO significantly decreased ADG and 42 d BW ($P < 0.05$), compared with SO; whereas BO significantly increased ADG and 42 d BW and decreased FCR ($P < 0.05$) compared with the RO. From 1 to 50 d of age, there was a trend of increase in ADG and 50 d BW ($P < 0.10$). From 1 to 21 d, there were no statistically significant differences ($P > 0.05$) in ADFI, ADG, FCR, or mortality rates between the treatments.

As shown in Table S8, compared with SO, dietary BO significantly increased half-eviscerated yield ($P < 0.05$). The study did not reveal any significant variations in the characteristics of carcass, eviscerated yield, breast muscle, leg muscle, and abdominal fat among the treatments ($P > 0.05$).

3.2. Meat quality

The data in Table 2 clearly indicate that there were no significant changes ($P > 0.05$) in $pH_{45\ min}$, $pH_{24\ h}$, L* value, b* value, drip loss, and shear force among SO, RO, LO, PO, and BO. Dietary BO resulted

Table 1
Effects of different dietary oil supplementation on growth performance of broiler chickens.[1,2]

Item	SO	RO	LO	PO	BO	SEM	*P*-value
1 to 21 d of age							
Initial body weight, g	43.53	43.56	43.58	43.57	43.56	0.032	0.913
BW at 21 d, g	366.20	372.39	380.47	373.60	373.95	3.121	0.766
ADFI, g	26.77	27.74	27.52	27.35	27.37	0.359	0.550
ADG, g/d	17.44	17.73	18.12	17.79	17.81	0.286	0.766
FCR	1.57	1.56	1.52	1.54	1.54	0.014	0.116
Mortality rate, %	0.00	1.11	0.00	1.67	0.00	0.476	0.183
22 to 42 d of age							
BW at 42 d, g	1014.31^{ab}	985.71^{c}	997.99^{ab}	1022.63^{ab}	1038.43^{a}	15.654	0.022
ADFI, g	72.86	70.80	71.80	73.46	72.30	0.843	0.303
ADG, g/d	30.98^{ab}	29.27^{c}	29.41^{ab}	30.90^{ab}	31.64^{a}	0.677	0.045
FCR	2.32^{ab}	2.42^{a}	2.44^{a}	2.39^{ab}	2.29^{b}	0.039	0.042
Mortality rate, %	2.22	3.39	2.78	2.24	1.11	1.331	0.594
43 to 50 d of age							
BW at 50 d, g	1345.12	1308.26	1325.15	1347.34	1378.36	19.211	0.077
ADFI, g	123.68	116.32	122.71	121.72	121.56	2.413	0.355
ADG, g/d	47.26	46.08	46.74	46.39	48.56	1.555	0.855
FCR	2.62	2.53	2.64	2.63	2.52	0.029	0.543
Mortality rate, %	0.60	1.79	0.00	1.73	1.67	0.913	0.622
1 to 50 d of age							
Initial body weight, g	43.50	43.50	43.50	43.50	43.50	nd	nd
BW at 50 d, g	1345.12	1308.26	1325.15	1347.34	1378.36	19.211	0.077
ADFI, g	74.44	71.46	74.01	74.18	73.74	0.947	0.287
ADG, g/d	26.03	25.44	25.63	26.08	26.70	0.182	0.078
FCR	2.86^{ab}	2.82^{b}	2.89^{a}	2.85^{ab}	2.73^{c}	0.012	0.007
Mortality rate, %	0.94	2.09	0.93	2.06	0.93	0.603	0.321

AFDI = average daily feed intake; ADG = average daily gain; FCR = feed to gain ratio; nd = not detected.
$^{a-c}$Means within a row with different superscript letters are significantly different ($P < 0.05$).
[1] Mean of 6 replicates (30 chickens per replicate) per treatment.
[2] SO, soybean oil group; RO, rice bran oil group; LO, lard group; PO, poultry oil group; BO, blended oil group.

Table 2
Effects of different dietary oil supplementation on meat quality of broiler chickens.[1,2]

Item	SO	RO	LO	PO	BO	SEM	*P*-value
$pH_{45\ min}$	6.09	6.00	6.01	6.09	6.08	0.082	0.752
$pH_{24\ h}$	5.68	5.68	5.57	5.70	5.66	0.056	0.367
$L^*_{45\ min}$	63.75	64.40	64.03	63.99	62.60	0.804	0.394
$a^*_{45\ min}$	13.24	13.46	13.98	13.55	14.89	0.475	0.147
$b^*_{45\ min}$	22.43	22.18	22.23	21.32	21.73	0.835	0.944
$L^*_{24\ h}$	67.64	67.25	68.35	67.65	65.63	1.012	0.344
$a^*_{24\ h}$	11.98^{c}	11.58^{c}	12.23^{c}	12.58^{bc}	14.02^{a}	0.438	0.002
$b^*_{24\ h}$	23.24	22.81	24.45	23.29	23.03	0.082	0.762
Drip loss, %	3.71^{ab}	4.29^{a}	3.65^{ab}	3.60^{ab}	3.14^{b}	0.307	0.042
Shear force, N	32.03	28.29	38.37	35.21	38.16	2.949	0.160
IMF, %	1.69^{b}	1.61^{b}	1.49^{b}	1.54^{b}	1.86^{a}	0.053	0.018
MDA, nmol/mL	2.10^{a}	2.28^{a}	2.32^{a}	2.19^{a}	1.43^{b}	0.085	0.001

L* = lightness; a* = redness; b* = yellowness; IMF = intramuscular fat; MDA = malonaldehyde.
[a-c]Means within a row with different superscript letters are significantly different ($P < 0.05$).
[1] Mean of 6 replicates (2 chickens per replicate) per treatment.
[2] SO, soybean oil group; RO, rice bran oil group; LO, lard group; PO, poultry oil group; BO, blended oil group.

in a higher a* value at 24 h and lower MDA content ($P < 0.05$), compared with SO.

3.3. Fatty acid composition

Fatty acid profiles of breast muscle are shown in Table 3. Compared with SO, C16:0 and C18:0, C16:1, C18:1 were significantly higher ($P < 0.05$), and C18:2, and C18:3 were significantly lower ($P < 0.05$) in RO, LO, PO, and BO. SFA in LO was significantly higher than that in SO, RO and BO ($P < 0.05$). Additionally, PUFA, n-6 and n-3 PUFA were the highest in SO, followed by BO, and significantly higher than those in other groups ($P < 0.05$). The n-6/n-3 ratio in RO was the highest, followed by LO, PO, and BO, but that in SO was the lowest ($P < 0.05$).

3.4. Untargeted lipidomic profile

Lipid profiling was conducted using Progenesis QI and the LIPID MAPS Structure Database. A total of 916 peak pairs were detected, including 666 for the UPLC–MS/MS data sets acquired in positive electrospray ionization (ESI+) mode and 250 in negative electrospray ionization (ESI-) mode. These lipid molecules can be further classified into 8 categories, consisting of 259 (28.28%) triglycerides (TG), 208 (22.71%) phosphatidylcholine (PC), 141 (15.39%) phosphatidyl ethanolamine (PE), 59 (6.44%) diglycerol (DG), 52 (5.68%) lysophosphatidyl choline (LPC), 49 (5.35%) sphingomyelin (SM), 32 (3.49%) dimethyl phosphatidyl ethanolamine (dMePE), 28 (3.06%) ceramide (Cer), 24 (2.62%) phosphatidylinositol (PI), 19 (2.07%) lysophosphatidyl ethanolamine (LPE), 13 (1.42%)

Table 3
Effects of different dietary oil supplementation on fatty acid composition of breast muscle in broiler chickens (% total fatty acids).[1]

Item	SO	RO	LO	PO	BO	SEM	*P*-value
C12:0	0.31^{b}	0.14^{c}	0.30^{b}	0.18^{c}	0.83^{a}	0.048	<0.001
C14:0	0.83^{c}	0.70^{c}	1.00^{b}	0.82^{c}	1.21^{a}	0.038	<0.001
C15:0	0.13	0.12	0.11	0.12	0.12	0.003	0.259
C16:0	23.06^{b}	25.62^{a}	26.22^{a}	25.34^{a}	25.64^{a}	0.289	0.001
C17:0	0.16	0.13	0.15	0.16	0.15	0.004	0.183
C18:0	6.10^{c}	5.85^{c}	8.73^{a}	6.75^{b}	6.58^{c}	0.093	0.001
C20:0	0.15^{c}	0.19^{ab}	0.15^{bc}	0.22^{a}	0.17^{bc}	0.006	0.002
C21:0	0.33^{d}	0.48^{b}	0.53^{a}	0.50^{ab}	0.40^{c}	0.015	<0.001
C22:0	0.26^{a}	0.21^{c}	0.21^{c}	0.23^{bc}	0.25^{ab}	0.006	0.001
SFA[2]	31.62^{c}	33.72^{b}	35.66^{a}	34.67^{ab}	31.36^{c}	0.344	<0.001
C14:1	0.16	0.17	0.18	0.17	0.20	0.007	0.205
C16:1	3.31^{b}	4.77^{a}	4.97^{a}	5.02^{a}	4.63^{a}	0.158	<0.001
C18:1	29.26^{c}	34.81^{b}	34.94^{a}	36.07^{a}	34.94^{a}	0.639	<0.001
MUFA[3]	32.72^{c}	39.75^{b}	40.09^{a}	41.26^{a}	40.37^{a}	0.766	<0.001
C18:2	28.80^{a}	22.17^{c}	16.54^{d}	18.14^{d}	24.13^{b}	0.857	<0.001
C18:3	2.17^{a}	0.87^{c}	0.67^{d}	0.77^{cd}	1.44^{b}	0.107	<0.001
C20:3	0.29^{bc}	0.29^{bc}	0.26^{c}	0.37^{a}	0.32^{b}	0.008	<0.001
C20:4	1.06^{a}	0.96^{a}	0.67^{b}	1.14^{a}	1.03^{a}	0.040	<0.001
C20:5	0.14^{b}	0.05^{d}	0.22^{a}	0.07^{cd}	0.09^{c}	0.012	<0.001
C22:6	0.24^{a}	0.23^{a}	0.11^{b}	0.25^{a}	0.19^{a}	0.014	0.002
PUFA[4]	32.70^{a}	24.56^{c}	18.46^{e}	20.74^{d}	27.19^{b}	0.972	<0.001
n-6 PUFA[5]	30.14^{a}	23.42^{c}	17.47^{e}	19.64^{d}	25.47^{b}	0.872	<0.001
n-3 PUFA[6]	2.56^{a}	1.15^{c}	0.99^{c}	1.09^{c}	1.72^{b}	0.114	<0.001
n-6/n-3 ratio	11.81^{d}	20.99^{a}	17.71^{b}	18.18^{b}	14.83^{c}	0.673	<0.001

SO = soybean oil group; RO = rice bran oil group; LO = lard group; PO = poultry oil group; BO = blended oil group.
[a-d]Means within a row with different superscript letters are significantly different ($P < 0.05$).
[1] Mean of 6 replicates (2 chickens per replicate) per treatment.
[2] SFA = C10:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0.
[3] MUFA = C14:1 + C15:1 + C16:1 + C17:1 + C18:1n9 + C20:1n9 + C22:1n9 + C24:1n9.
[4] PUFA = C18:2n6 + C20:2 + C18:3n6 + C18:3n3 + C20:3n3 + C20:3n6 + C20:5n3 + C22:2 + C22:6n3.
[5] n-6 PUFA = C18:2n6 + C20:3n6 + C20:4n6.
[6] n-3 PUFA = C18:3n3 + C20:3n3 + C20:5n3 + C22:6n3.

phosphatidylglycerol (PG), and 32 (3.51%) others (Fig. S1). The PLS-DA model was used to analyze the lipidome data generating different cuts of SO, LO, and BO breast muscle samples (Fig. 1A and B). The R2 and Q2 values in positive ion mode are 0.733 and 0.546 (Fig. 1C), respectively, and those in negative ion mode are 0.440 and 0.289 (Fig. 1D), respectively, indicating that the model is stable and able to explain and predict sample differences. The lipids having PLS-DA variable importance in projection (VIP) > 1, fold change (FC) > 2.0 or < 0.5 and ANOVA $P < 0.05$ were considered to have significant potential for separation of the 3 different breast muscles. A total of 136 lipid metabolites with significant differences were selected under ESI+ and ESI- ion modes, including 33 PC, 29 TG, 22 PE, 11 dMePE, 11 LPC, 6 LPE, 6 PG, 5 SM, 4 PI, 2 ceramide, 2 PS, 2 DG, 1 LdMePE, 1 LPG, and 1 PIP (Fig. S1B). The heatmap of the top 20 differential lipid metabolites is shown in Fig. 1E. There were 12 TG, 2 PE, 3 LPC, 1 PI, 1 SM and 1 PC. Blended oil significantly increased 10 TG in TG (12:0/18:2/18:3) + Na, TG (15:1/16:0/18:1) + NH_4, TG (16:0/10:0/16:0) + Na, TG (16:0 12:0/12:0) + Na, TG (16:0/12:0/

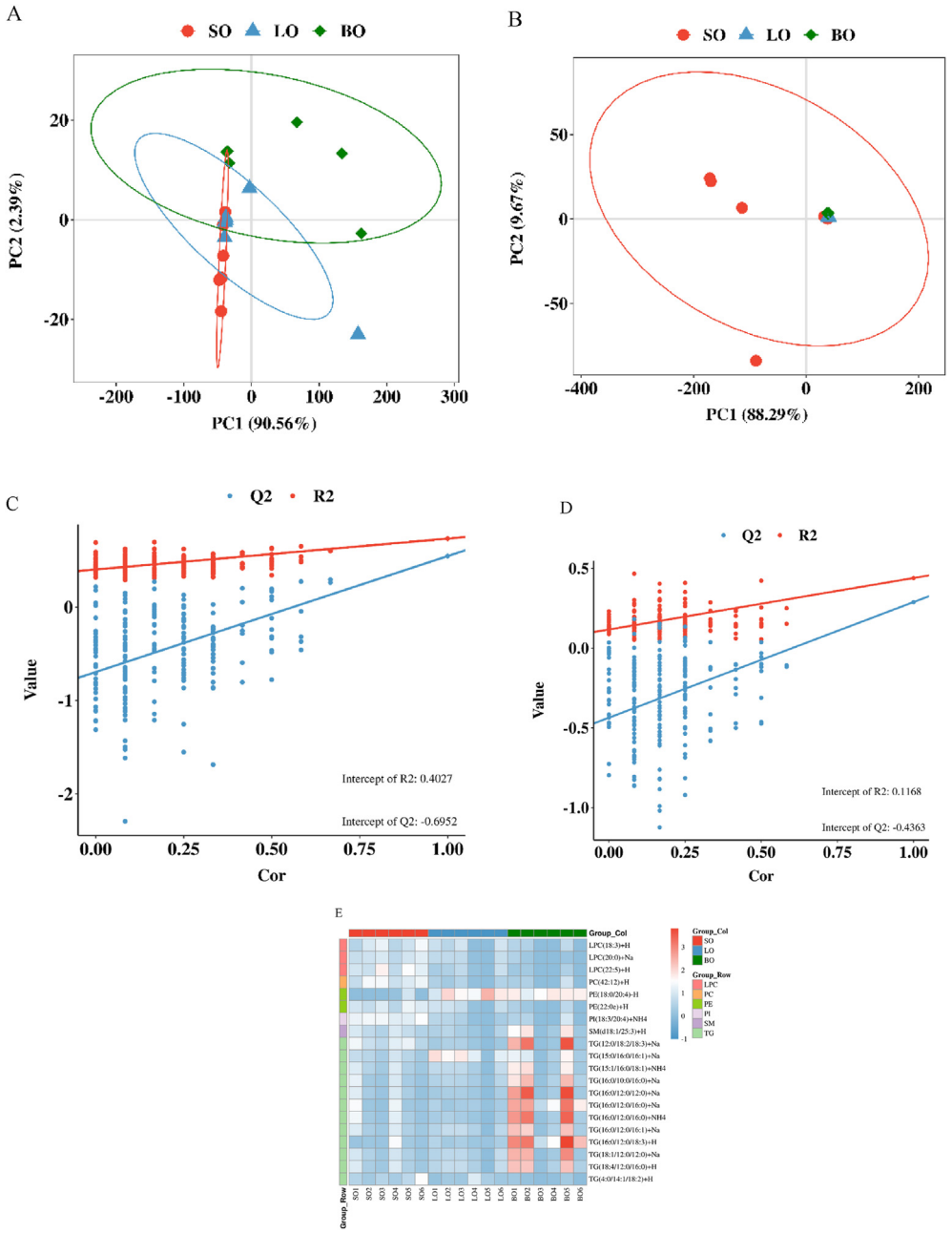

Fig. 1. Effects of different dietary oil supplementation on lipid metabolites of breast muscle in broiler chickens ($n = 6$). (A) Partial least squares discriminant analysis (PLS-DA) score of lipid metabolites in positive ion mode; (B) PLS-DA score of lipid metabolites in negative ion mode; (C) lipid metabolite replacement test diagram in positive ion mode; (D) lipid metabolite replacement test diagram in negative ion mode; (E) abundance of top 20 differential lipid metabolites by variable importance in the projection (VIP) ranking. SO, soybean oil group; LO, lard group; BO, blended oil group. LPC = lysophosphatidyl choline; PC = phosphatidylcholine; PE = phosphatidyl ethanolamine; PI = phosphatidylinositol; SM = sphingomyelin; TG = triglycerides.

16:0) + Na, TG (16: 0/12:0/16:0) + NH_4, TG (16:0/12:0/16:1) + Na, TG (16:0/12:0/18:3) + H, TG (18:1/12:0/12:0) + Na, and TG (18:4/12:0/16:0) + H.

3.5. Compositions of volatile organic compounds

Volatile organic compounds in breast muscle are shown in Table 4. An obvious separation among the samples of SO, LO, and BO based on PCA is shown in Fig. 2. The volatolomics identified a total of 41 VOC, including 5 aldehydes, 3 alcohols, 2 ketones, 8 esters, 21 alkanes and 2 heterocyclic compounds in breast muscle. The contents of hexanal, octanal, nonanal, and total aldehyde in SO were significantly higher than those in LO and BO ($P < 0.05$). The contents of 1-octen-3-ol,2-ethyl-1-hexanol and total alcohol in SO were significantly higher than those in LO and BO ($P < 0.05$). The contents of dodecanoic methyl ester, decanoic methyl ester, and total esters in BO were significantly higher than those in SO and LO ($P < 0.05$). The contents of heptadecane and total alkanes in LO were significantly higher than those in SO and BO ($P < 0.05$). In addition, the contents of total heterocyclic substances in SO were significantly higher than those in LO and BO ($P < 0.05$).

3.6. Gut microbiota structure

As shown in Table S9, there were no significant differences in α-diversity including Species observed, Shannon, Simpson, ACE, Chao, and Good's coverage among SO, LO, and BO lipid treatments ($P > 0.05$). Distinct clustering of microbiota in each treatment was exposed by PCoA (Fig. 3A). As shown in Fig. 3B, Firmicutes, Bacteroidota, Campilobacterota, Cyanobacteria, Proteobacteria, Verrucomicrobiota, Actinobacteria, Synergistota, and others were the top 10 major phyla by relative abundance. At the phylum level, BO increased Firmicutes and Firmicutes to Bacteroidota ratio ($P < 0.05$), but decreased Campylobacterota, Cyanobacteria, and Proteobacteria ($P < 0.05$), compared with SO and LO. Figure 3C displays the top 20 primary genera and their relative abundances across all

Table 4
Effects of different dietary oil supplementation on volatile organic compounds (VOC) composition of breast muscle in broiler chickens.[1]

No.	VOC	RT	SO	LO	BO	SEM	*P*-value
1	Hexanal	1.079	0.94^{a}	0.25^{b}	0.27^{b}	0.013	0.001
2	Octanal	15.522	0.30^{a}	0.15^{b}	0.16^{b}	0.022	<0.001
3	Nonanal	25.532	2.20^{a}	1.41^{b}	1.25^{b}	0.131	<0.001
4	Benzaldehyde	11.708	2.81	2.23	2.32	0.108	0.063
5	Benzeneacetaldehyde	19.337	0.70^{b}	1.34^{a}	1.19^{a}	0.089	0.007
	Total aldehyde		6.95^{a}	5.37^{b}	5.19^{b}	0.282	0.009
6	1-Octen-3-ol	13.727	4.02^{a}	1.80^{b}	1.98^{b}	0.276	<0.001
7	2-Ethyl-1-hexanol	18.370	2.28^{a}	0.54^{b}	0.72^{b}	0.271	0.006
8	1-Decanol	50.004	0.35	0.41	0.38	0.022	0.487
	Total alcohol		6.64^{a}	2.74^{b}	3.08^{b}	0.522	<0.001
9	4-Octanone	12.631	0.50^{a}	0.15^{b}	0.18^{b}	0.048	0.001
10	1-(3,5-Dimethylpyrazinyl)- ethanone	31.999	0.41^{b}	0.88^{ab}	1.04^{a}	0.102	0.021
	Total ketones		0.91	1.04	1.21	0.083	0.319
11	Hexanoic acid, methyl ester	8.899	2.81^{a}	1.40^{b}	1.25^{b}	0.221	0.001
12	Octanoic acid, methyl ester	27.451	1.28^{a}	0.68^{b}	1.26^{a}	0.088	0.003
13	Decanoic acid, methyl ester	45.151	0.81^{b}	0.71^{b}	1.76^{a}	0.163	0.007
14	Dodecanoic acid, methyl ester	57.190	2.19^{b}	1.15^{b}	8.82^{a}	1.033	<0.001
15	Sulfurous acid,2-propyl tetradecyl ester	58.352	0.66	0.83	0.67	0.052	0.358
16	Methyl tetradecanoate	60.940	0.49^{b}	0.82^{a}	0.88^{a}	0.051	0.001
17	Hexadecanoic acid, methyl ester	62.785	11.43^{b}	18.68^{a}	16.13^{a}	0.980	0.002
18	Methyl stearate	64.228	0.02^{b}	0.07^{a}	0.07^{a}	0.012	<0.001
	Total esters		19.70^{b}	24.34^{ab}	30.85^{a}	1.541	0.004
19	Octane	0.946	0.71^{a}	0.17^{b}	0.15^{b}	0.079	0.001
20	(E)-5-Undecene	23.899	0.46^{a}	0.19^{b}	0.32^{ab}	0.052	0.048
21	Undecane	24.937	3.78^{a}	1.18^{b}	1.31^{b}	0.374	0.001
22	3-Methyl-undecane	31.770	0.16^{a}	0.05^{b}	0.05^{b}	0.022	0.004
23	Dodecane	34.686	0.71^{a}	0.35^{b}	0.33^{b}	0.062	0.001
24	Heptacosane	41.066	1.43^{a}	0.61^{b}	0.62^{b}	0.123	<0.001
25	Tridecane	43.256	2.13^{a}	1.36^{b}	1.96^{a}	0.112	0.005
26	2,6,10-Trimethyl-dodecane	45.785	0.57^{a}	0.34^{b}	0.35^{b}	0.034	0.001
27	9-Methyl-nonadecane	46.042	0.78	0.56	0.40	0.083	0.135
28	10-Methyl-eicosane	48.428	4.30	3.69	3.51	0.216	0.323
29	Tetradecane	50.561	0.44	0.46	0.45	0.024	0.952
30	Tetratetracontane	54.261	0.37^{a}	0.40^{a}	0.18^{b}	0.042	0.017
31	Pentadecane	56.175	1.41	1.00	0.90	0.133	0.275
32	3-Methyl-pentadecane	58.514	2.02	2.82	2.88	0.178	0.093
33	Hexadecane	59.147	0.60	0.60	0.54	0.033	0.654
34	2,6,10-Trimethylpentadecane	59.914	3.33	5.22	4.09	0.341	0.063
35	Heptadecane	60.641	10.87^{b}	16.29^{a}	12.79^{b}	1.113	0.013
36	Octadecane	61.707	2.68	3.79	2.92	0.212	0.064
37	2,6,10,14-Tetramethyl-hexadecane	61.768	5.24	8.66	5.80	0.653	0.060
38	Nonadecane	62.572	2.81	4.04	3.00	0.262	0.113
39	Eicosane	63.314	0.03	0.05	0.03	0.002	0.057
	Total alkanes		44.80^{b}	51.82^{a}	42.57^{b}	1.903	0.012
40	2-Pentyl-furan	14.080	7.97^{a}	3.69^{b}	3.46^{b}	0.696	0.004
41	2,6-Diethyl-pyrazine	22.418	0.42^{b}	1.04^{a}	0.85^{ab}	0.102	0.021
	Total heterocyclic compounds		8.39^{a}	4.74^{b}	4.30^{b}	0.633	0.006

SO = soybean oil group; RO = rice bran oil group; LO = lard group; PO = poultry oil group; BO = blended oil group.
^{a,b}Means within a row with different superscript letters are significantly different ($P < 0.05$).
[1] Mean of 6 replicates (2 chickens per replicate) per treatment.

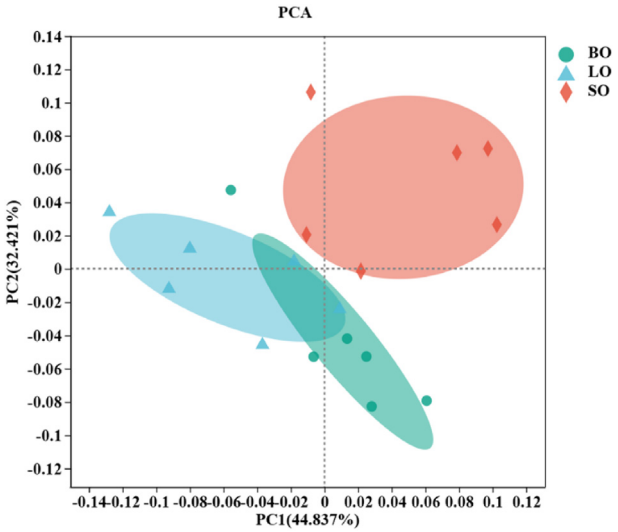

Fig. 2. Score plots of principal component analysis derived from GC–MS data showing the volatile organic compounds (VOC) differences of breast muscle samples among different treatment groups of broiler chickens. SO, soybean oil group; LO, lard group; BO, blended oil group.

groups. Compared with SO, BO increased *Ruminococcus_torques and Christensenellaceae_R-7* and decreased *Phascolarctobacterium, norank_f__norank_o__Clostridia_vadinBB60,* and *norank_f_norank_o_Gastranaerophilales* ($P < 0.05$). LO had higher *Faecalibacterium* and *Megamonas* compared with SO and BO ($P < 0.05$).

3.7. mRNA expression of genes related to lipid metabolism in breast muscle

As shown in Fig. 4, BO increased the transcript abundance of peroxisome proliferator activated receptor alpha (*PPARα*), acyl-CoA oxidase 1 (*ACOX1*), and carnitine palmitoyl-transferase 1 (*CPT1A*) in breast muscle compared to SO ($P < 0.05$). There were no differences in the transcript abundance of peroxisome proliferator activated receptor gamma (*PPARγ*), fatty acid binding protein 3 (*FABP3*) and acyl-CoA synthetase long-chain family member 5 (*ACSL5*) among the treatments ($P > 0.05$).

3.8. Correlations between breast muscle metabolites, volatile organic compounds, and microbial communities

Pearson's correlations testing associations between lipid metabolites, VOC, and the primary intestinal bacterial genera revealed that the relative abundance of *g_Lactobacillus* and *Christensenellaceae_R-7* were positively correlated with the content of TG (16:0/12:0/16:0) + Na, TG (16:0/12:0/18:3) + H, TG (18:1/12:0/12:0) + Na, TG (16:0/12:0/16:1) + Na, TG (18:4/12:0/16:0) + H, TG (16:0/10:0/16:0) + Na, TG (16:0/12:0/12:0) + Na, TG (16:0/12:0/16:0) + NH_4, TG (12:0/18:2/18:3) + Na, and TG (15:1/16:0/18:1) + NH_4 in breast muscle (Fig. 5A). In addition, the relative abundance of *Megasphaera* was positively correlated with hexanal, 2-ethyl-1-hexanol, 4-octanone, (E) -5-undecene ($P < 0.05$), and 3-methyl-undecane, octane and undecane ($P < 0.01$). The relative abundance of both *Lactobacillus* and *R-7 Christensenaceae* were positively correlated with methyl laurate ($P < 0.05$). *Campylobacter* was positively correlated with 3-methyl-undecane, undecane, octane and tetradecane ($P < 0.01$) (Fig. 5B).

4. Discussion

Lipids are important constituents in poultry diets, and they are high density forms of dietary energy density and improve the productive performance of chickens (Saleh et al., 2021; Song et al., 2022). Fat and oils differ in their fatty acid composition, which may be responsible for the different effects they may have on chickens. It has been reported that replacing dietary SO with RO improves growth performance in chickens (Selim et al., 2021). Furthermore, it has been demonstrated that feeding chicken diets supplemented with PO instead of SO reduced abdominal fat deposition without changing growth performance and carcass characteristics (Saleh et al., 2021). In performance and carcass traits measurements, FCR alone was improved when dietary corn oil was replaced with coconut oil in chickens (Kim et al., 2020). In the present study, there was a trend toward improved growth of chickens feeding diets containing PO or BO, but not RO, which is consistent with the previous studies mentioned above. Previous studies have suggested that BO generate better weight gain in chickens than a single oil (Long et al., 2018; Wang et al., 2013; Zhong et al., 2014). It has been revealed that diets supplemented with flaxseed oil and palm oil increase the average daily gain in chickens compared with lard, flaxseed oil and palm oil (Zhong et al., 2014). A previous study showed that adding mixed oils (15% corn oil, 10% coconut oil, 15% flax oil, 20% palm oil, 15% peanut oil, and 25% soybean oil) improved growth performance in chickens (Long et al., 2018). In the present experiment, chickens fed the diet containing BO increased average daily gain and reduced feed to gain ratio from 22 to 42 d of age. These results indicated that BO may have a more balanced fatty acid composition, in terms of improvement in the utilization efficiency of fatty acids in chickens. The favorable effect may be due to fatty acid composition of the fat source (Deng et al., 2022; Rodriguez-Sanchez et al., 2019).

Objective indices of meat quality include meat color, pH, drip loss and shear force. Meat color is the main index reflecting muscle appearance and water-holding capacity. A lower L* value means darker meat color, a higher a* value means higher redness, and higher b* value means higher yellowness. It has been reported that broilers fed mixed oil had increased a* value in thigh muscle, as well as a decrease in drip loss in both the breast and thigh muscles (Long et al., 2018). Previous research showed that broilers fed glycerol monolaurate could maintain meat color (Valentini et al., 2020). Furthermore, chickens fed diets supplemented with antioxidants showed higher deposition of antioxidants in their meat, which caused enhanced meat redness through reducing lipid and protein oxidation (Adeyemi, 2021). The mechanism for improved meat color involves the fact that glycerol monolaurate has direct and indirect antioxidant action leading to decreased meat lipid peroxidation. The present experiment showed that dietary provision of BO increased 24 h a* value and decreased content of MDA in breast muscle, which may be due to increased content of MCFA in palm and coconut oil and its deposition into muscle, and it in turn increases anti-oxidative stability and reduces the oxidation of myoglobin, thereby maintaining meat color.

The fatty acid composition of the diet is the most significant factor that determines the profile of fatty acids in the tissue of chickens (Cortinas et al., 2004). A diet supplemented with flax oil increased n-3 PUFA in breast muscle, while the addition of corn oil increased n-6 PUFA (Kanakri et al., 2018). Similar results were also obtained where dietary supplementation with palm oil increased C18:1 and MUFA in breast muscle (Khatun et al., 2017). In the present study, the SO diet increased C18:2 and C18:3 PUFA in the breast muscle, while LO, PO diets increased C16:0 and C18:0 and BO diets increased C16:1 and C18:1, indicating that the fatty acid composition of chicken meat reflects dietary fatty acid

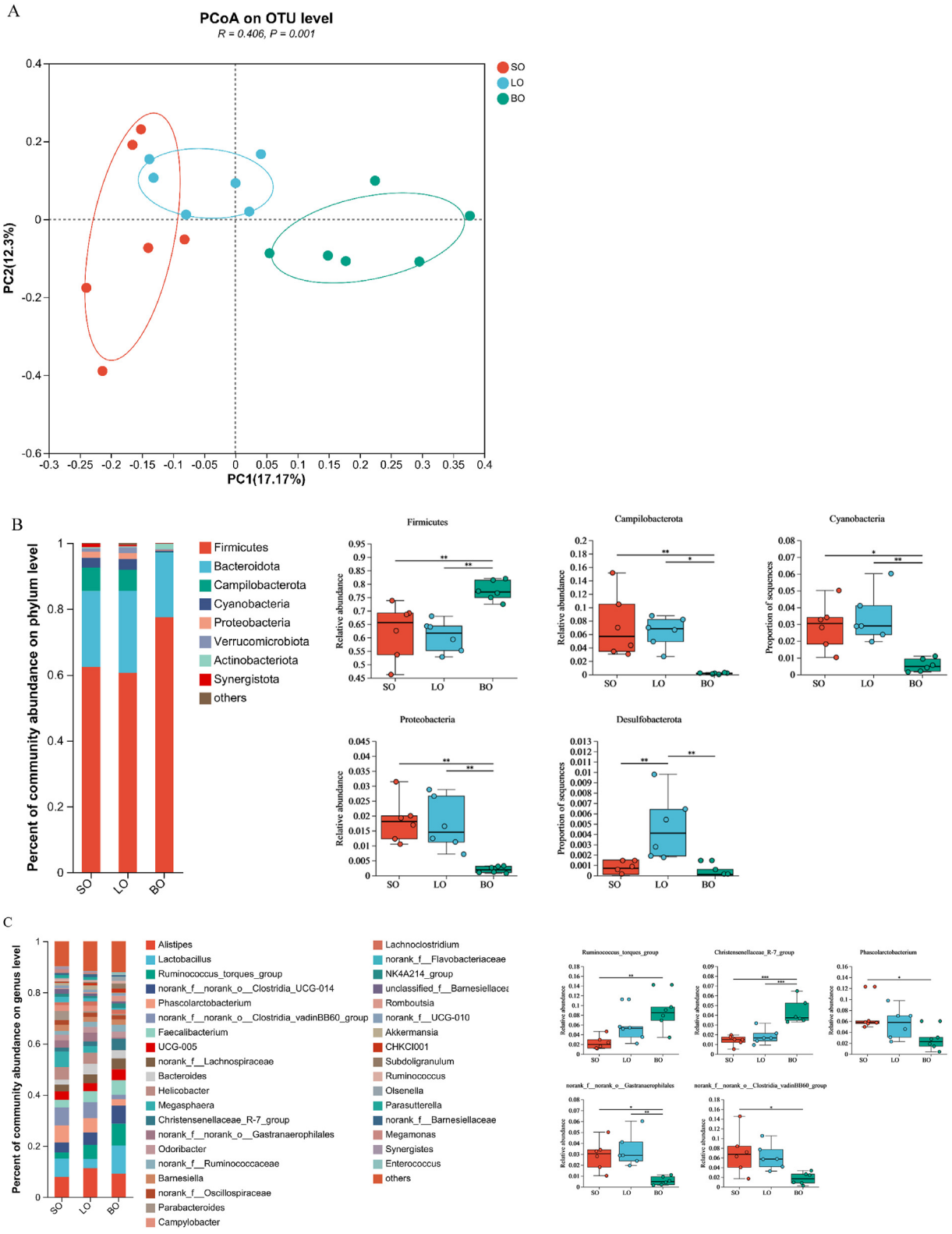

Fig. 3. Effects of different dietary oil supplementation on the gut microbiota of broiler chickens ($n = 6$). (A) Principal coordinate analysis (PCoA) at the OTU level. (B) Stacked bar chart representing the relative abundance of colonic bacteria at the phylum level (top 10) in the different dietary groups. (C) Stacked bar chart representing the relative abundance of colonic bacteria at the genus level (top 20) in the different dietary groups. OTU = operational taxonomic unit. SO, soybean oil group; LO, lard group; BO, blended oil group. $^{*}P < 0.05$; $^{**}P < 0.01$, $^{***}P < 0.001$.

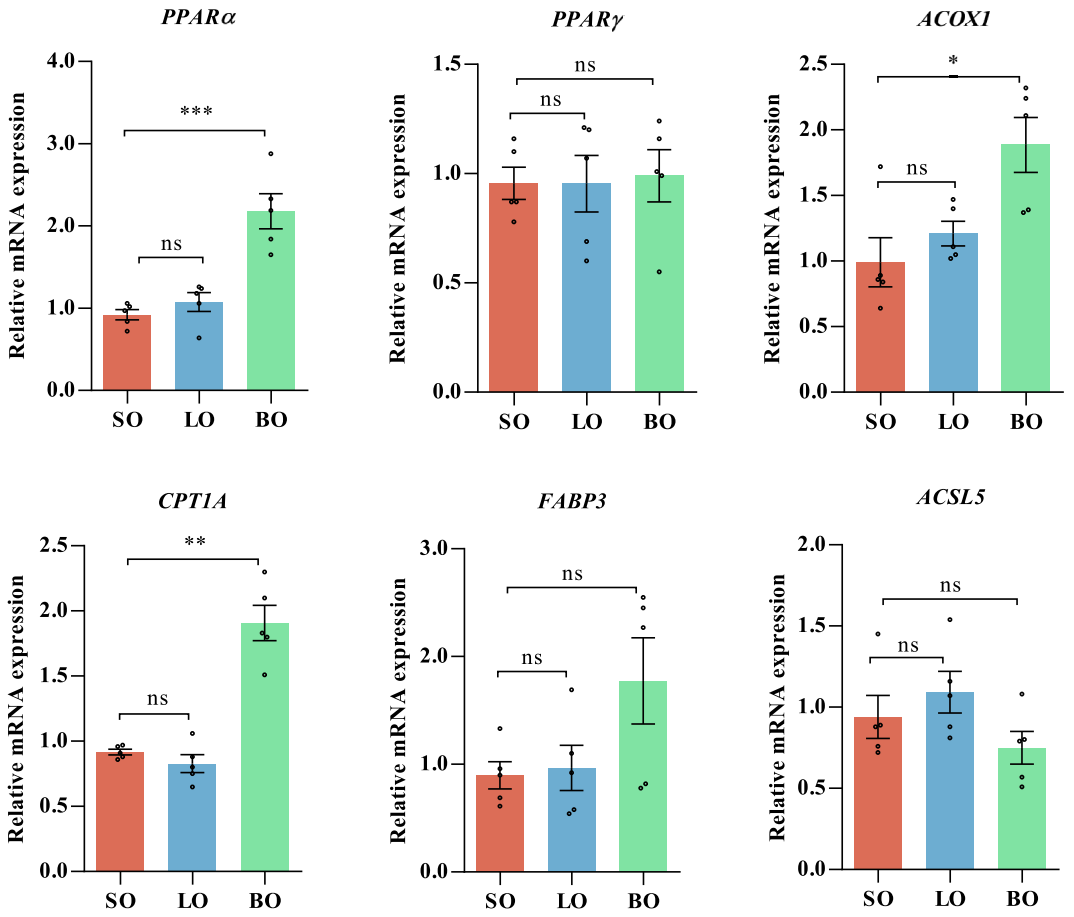

Fig. 4. Effects of different dietary oil supplementation on the relative gene expression of breast muscle in broiler chickens ($n = 6$). SO, soybean oil group; LO, lard group; BO, blended oil group. *PPARα* = peroxisome proliferator-activated receptor α; *PPARγ* = peroxisome proliferator-activated receptor γ; *ACOX1* = acyl-CoA oxidase 1; *CPT1A* = carnitine palmitoyltransferase 1; *FABP3* = fatty-acid binding protein 3; *ACSL5* = acyl-CoA synthetase long chain family member 5; ns = not significant. *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$.

composition. As an essential nutrient, unsaturated fatty acids are also important precursors of chicken meat flavor (Dinh et al., 2021; Han et al., 2023; Khan et al., 2015; Zhao et al., 2017). It has been found that C16:1 and C18:1 were positively correlated with meat flavor, flavor preference, and overall acceptability. However, PUFA such as C18:2 and C18:3 were negatively correlated with the flavor (Cameron et al., 2000) because C18:2 and C18:3 affect volatile lipid oxidation products in the Maillard reaction during roasting, which directly or indirectly affect meat flavor (Alfaia et al., 2019).

Lipidomics is a means of large-scale high-throughput sequencing of lipid metabolites in samples, and this technique greatly increases the breadth and depth of investigation of meat lipids (Chiesa et al., 2022). We selected SO, LO and BO as references to analyze the lipid structure in breast muscle. In this study we found that TG (16:0/12:0/16:0) + Na, TG (16:0/12:0/18:3) + H, TG (16:0/10:0/16:0) + Na, TG (16:0 12:0/12:0) + Na, and TG (16:0/12:0/16:0) + NH_4 in breast muscle were positively correlated with the proportion of SFA, while PC (42:12) + H and PI (18:3/20:4) + NH_4 were positively correlated with the proportion of PUFA such as C18:2, C18:3 and C22:6. We also found that most of the top 20 lipid molecules in breast were TG, and the relative abundance of TG in meat from BO was generally higher than that in meat from birds fed SO and LO. The triglycerides TG (12:0/18:2/18:3) + Na, TG (16:0/12:0/12:0) + Na, TG (16:0/12:0/16:0) + Na, TG (16:0/12:0/16:0) + NH_4, TG (16:0/12:0/16:1) + Na, TG (16:0/12:0/18:3) + H, TG (18:1/12:0/12:0) + Na, TG (18:4/12:0/16:0) + H all contain C12:0, which may be due to the high content of methyl laurate in BO. Triglycerides are deposited as a caloric store (Aleidi et al., 2022) which are mobilized, or simply oxidized when energy supply is insufficient. The synthesis and decomposition of TG play a crucial role in the energy metabolism of muscle (Liu et al., 2019). Deposition of TG was higher in birds fed BO, which may indicate that more TG from balanced oil accurately deposited in muscle, rather than simply playing a role in energy supply. As the main component of IMF, TG derives from the synthesis or the de novo synthesis of fatty acids in the circulatory system and plays an essential role in muscle lipid deposition and meat quality improvement. It is closely related to the expression of lipid metabolism-related genes such as *PPARs*, *FABPs*, *LPL*, *SCD*, *ACSL1*, *ACOX1*, and *SLC16A7* (Liu et al., 2019; Knight et al., 2019; Wang et al., 2022). Positive correlation exists between *PPARα* expression and IMF in both breast and thigh muscle (Liu et al., 2022; Wang et al., 2022; Ye et al., 2014). Activation of *PPARα* upregulates the expression of genes involved in fatty acid synthesis and lipid metabolism (*CPT1* and *ACOX1*) and enhances IMF deposition in muscle (Chen et al., 2022). In the present study, there was substantial increment in the relative abundance of *PPARα*, *ACOX1* and *CPT1A* transcripts in breast of chickens fed BO. More extensive investigation is warranted for creating a better understanding of how BO in chicken diets increases synthesis of fatty acids and promotes synthesis and deposition of TG in muscle.

The complex populations of gut microbiota harbored by individuals have impact on nutrient utilization, growth and health (Yadav and Jha, 2019). In the present study, PCoA and ANOSIM analysis revealed that chickens fed a diet supplemented with BO had a different composition of the cecal microbiota, distinct from that in SO and LO. Compared with the controls, there were more

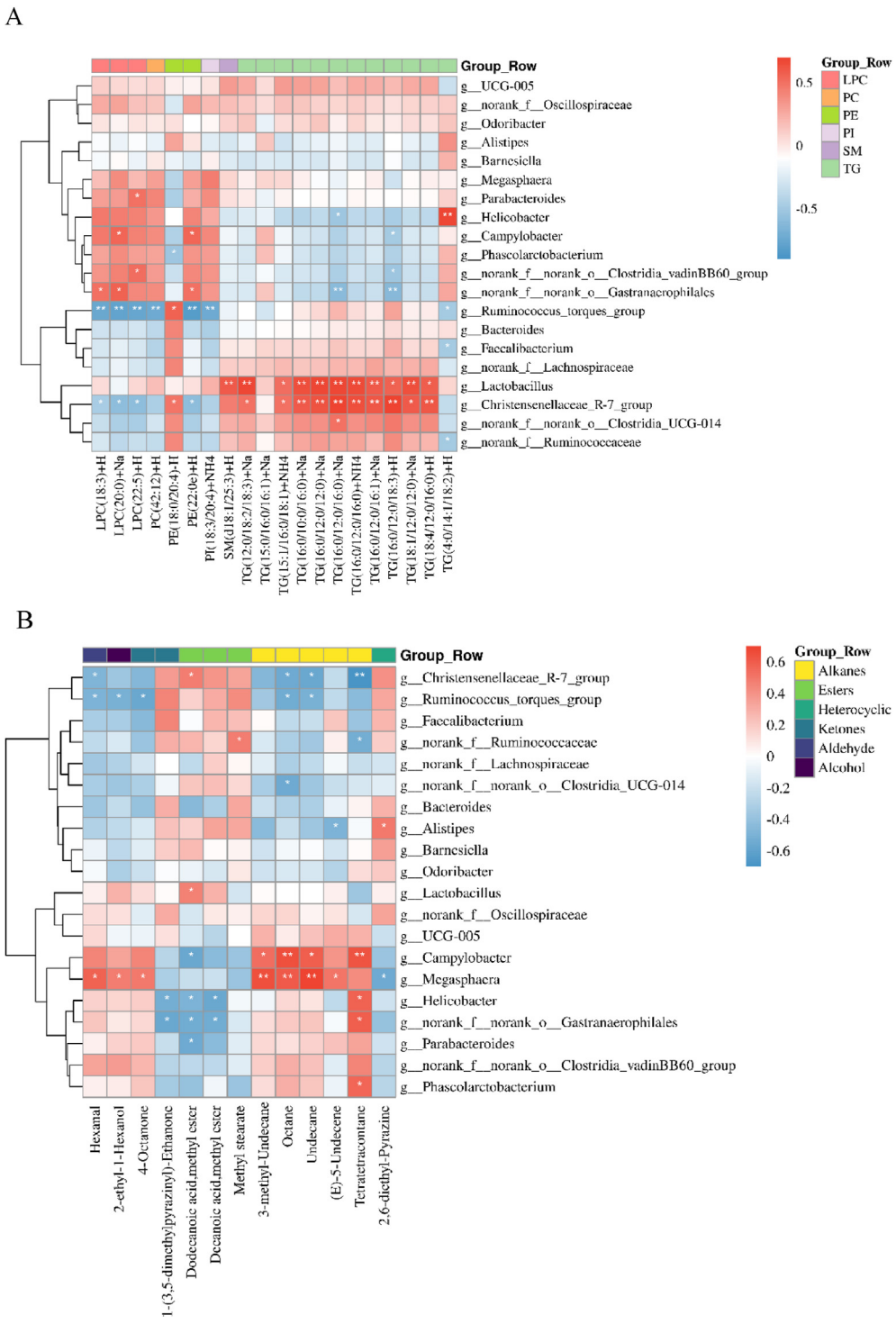

Fig. 5. Correlations between breast muscle metabolites, volatile organic compounds, and microbial communities. (A) Correlation heat map between intestinal microbial community (top 20 at genus level abundance) and breast muscle differential lipid metabolites (top 20 at significant difference). (B) Correlation heat map between gut intestinal microbial communities (top 20 at genus level abundance) and breast muscle VOC (VIP > 1). *$P < 0.05$; **$P < 0.01$.

Firmicutes and less Proteobacteria and Campilobacterota. Firmicutes, as the main dominant bacterial community in the cecum, accounted for more than 80% of the overall microbial community. Firmicutes contribute to the synthesis of short-chain fatty acids such as butyrate and propionic acid, which play many beneficial roles in the intestine, such as serving as an energy supply through gluconeogenesis, reducing undesirable flora in the cecum, stimulating the proliferation and differentiation of intestinal epithelial cells, and thus increasing the absorption and utilization of nutrients in the intestine (Sun et al., 2022). At the genus level, BO increased *Ruminococcus_torques* and *Christensenellaceae_R-7* and decreased *Phascolarctobacterium*. *Ruminococcus_torques*, a subgroup of *Ruminococcus*, which is capable of producing butyrate and other SCFA through glucose metabolism and cellulose degradation (Crost et al., 2023). A previous study reported that *Ruminococcus_torques* was one of the important bacterial genera for predicting IMF deposition

(Wen et al., 2023). *Christensenellaceae_R-7* is a newly discovered microbial community and it has been shown to play an important role in intestinal health (Tavella et al., 2021; Waters and Ley, 2019). Studies have shown that *Christensenellaceae_R-7* can improve the development of gastrointestinal tract, thereby improving the digestion and absorption capacity of nutrients in animals (Ma et al., 2022).

In addition, studies have shown that certain intestinal bacteria can increase flavor precursors in muscle by regulating lipid metabolism, thus affecting meat flavor. Studies have shown that the relative abundance of intestinal *UCG-010_unclassified* is closely related to the production of nonanal, (E, E)-2, 4-nonadienal, total aldehyde and inosine monophosphate in pork, which help improve flavor of pork (Liu et al., 2023). *Lactobacillus reuteri 1* may improve pork flavor by increasing inosinic acid and glutamic acid (Tian et al., 2021). Dietary *Clostridium butyricum* supplementation resulted in higher levels of total aldehydes, alcohols, ketones, furans, and sulfur-containing compounds in chicken breast muscle (Liu et al., 2017). It has been reported that flavor precursors are affected by the structure of intestinal flora. For example, PUFA such as C20:5 and C22:6 in muscle were positively correlated with the abundance of intestinal *Bifidobacterium* and *Lactobacillus* (Wang et al., 2017). In the present study, *g_Lactobacillus* and *Christensenellaceae_R-7* showed strong positive correlation with TG, which may indicate that *g_Lactobacillus* and *Christensenellaceae_R-7* could change the lipid structure of chicken meat by promoting deposition of TG. Furthermore, the relative abundance of *g_Lactobacillus* and *Christensenellaceae_R-7* was positively correlated with the proportion of methyl laurate. These results suggest that the gut microbial community may regulate lipid metabolism and further influence the formation of volatile flavor compounds in chicken breast muscle.

5. Conclusions

The present study showed that dietary BO could improve meat quality, and modulate gut microbiota. Blended oil accelerated glyceryl phospholipid deposition and total ester flavor substances formation, but inhibited the deposition of heterocyclic compounds. Genera *g_Lactobacillus* and *Christensenellaceae_R-7* may influence the formation of volatile flavor compounds in chicken breast muscle. These results suggest that the potential beneficial effects of BO may be associated with the regulation of lipid metabolism and gut microbiota structure.

Credit Author Statement

Dong Ruan: Conceptualization, Methodology, Data curation, Formal analysis, Original draft writing, Funding acquisition. **Jiashuai Jiang:** Data curation, Formal analysis, Validation. **Wenjie Huang**: Data curation, Formal analysis, Validation. **Ahmed Mohamed Fouad** and **Hebatallah Kasem El-Senousey:** Methodology, Data curation, Validation, Writing-review & editing. **Xiajing Lin** and **Sai Zhang:** Methodology, Data curation, Validation. **Lihua Sun**, **Shijuan Yan** and **Zongyong Jiang:** Supervision. **Shouqun Jiang:** Conceptualization, Funding acquisition, Methodology.

Declaration of competing interests

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix supplementary data

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