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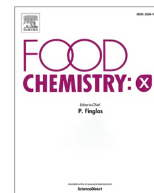

Dietary riboflavin supplementation improves meat quality, antioxidant capacity, fatty acid composition, lipidomic, volatilomic, and proteomic profiles of breast muscle in Pekin ducks

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ABSTRACT

Our objective was to determine effects of supplemental dietary riboflavin on meat quality, antioxidant capacity, fatty acid composition, lipidomic, volatilomic, and proteomic profiling of duck breast muscle. The results showed that dietary riboflavin supplementation significantly increased growth performance, breast meat yield, intramuscular fat content, polyunsaturated fatty acid (PUFA), n3-PUFA, n6-PUFA, redness (a*), and pH_{24h}, but decreased lightness (L*) and yellowness (b*). Furthermore, riboflavin supplementation significantly improved muscle antioxidant capacity based on various biochemical parameters. Lipidomic and volatilomic analyses revealed that riboflavin supplementation markedly increased breast meat phosphatidylglycerol and coenzyme Q contents and two favourable key odorants, citronellyl acetate and 3-(methylthio)-propanal. Proteomics analyses confirmed that riboflavin supplementation activated mitochondrial aerobic respiration, including fatty acid beta oxidation, the tricarboxylic acid cycle, and oxidative phosphorylation. In conclusion, supplementing duck diets with riboflavin enhanced breast meat quality, attributed to increases in antioxidant capacity and mitochondrial functions.

1. Introduction

Duck meat is popular globally, due to favourable nutritional characteristics, including high protein, low fat, and relatively high concentrations of polyunsaturated fatty acids (PUFA) (Khan et al., 2019; Jin et al., 2021). However, these characteristics, particularly high PUFA, make duck meat is susceptible to oxidation (Yu et al., 2021). Oxidation of lipids or proteins in meat reduces its quality, including color, flavour, and nutritional value (Falowo et al., 2014). However, antioxidant capacity and meat quality have been improved by supplementing diets with antioxidants, such as vitamin C (Skřivan et al., 2012), vitamin E (Bellés et al., 2018), sodium butyrate and vitamin D_3 (Gao et al., 2022), resveratrol (Jin et al., 2021), and riboflavin (Jiang et al., 2019).

Riboflavin, a natural nutrient and a precursor of two coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), is involved in many metabolic processes such as antioxidant systems, fatty

Abbreviations: ADG, average daily weight gain; ADFI, average daily feed intake; CON, control; CoQ, coenzyme Q; DG, diglyceride; FA, fatty acids; FAD, flavin adenine dinucleotide; FC, fold change; FDR, false discovery rate; FMN, flavin mononucleotide; GSH, reduced glutathione; GR, glutathione reductase; HPLC, high performance liquid chromatography; IMF, intramuscular fat; iTRAQ, isobaric tags for relative and absolute quantification; KEGG, Kyoto Encyclopedia of Genes and Genomes; LPC, lyso-phosphatidylcholine; LPC-O, lyso-plasmanyl-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; LPE-P, lyso-plasmenyl-phosphatidylethanolamine; LPG, lyso-phosphatidylglycerols; LPI, lyso-phosphatidylinositols; LPS, lyso-phosphatidylserines; MDA, malondialdehyde; MG, monoacylglycerol; MUFA, monounsaturated fatty acids; OPLS-DA, orthogonal partial least squares discriminate analysis; PA, phosphatidic acids; PC, phosphatidylcholine; PC-O, plasmanyl-phosphatidylcholine; PE, phosphatidylethanolamine; PE-P, plasmenyl-phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositols; PUFA, polyunsaturated fatty acid; PS, phosphatidylserines; PSE, pale, soft and exudative; SL, saccharolipids; ST, sterol lipids; T-AOC, total antioxidant capacity; TCA, tricarboxylic acid; TG, triglyceride; T-SOD, total superoxide dismutase; UFA, unsaturated fatty acids.

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acid oxidation, tricarboxylic acid (TCA) cycle, and the electron transport chain (Saedisomeolia and Ashoori, 2018; Suwannasom et al., 2020; Olfat et al., 2022). In mammals and poultry, riboflavin deficiency causes growth depression, dermatitis, glossitis, and anaemia (Tang et al., 2015, 2017, 2019; Cogburn et al., 2018; Saedisomeolia and Ashoori, 2018; Olfat et al., 2022). Furthermore, as riboflavin has essential roles in antioxidant systems, its deficiency leads to oxidative stress in rats (Levin et al., 1990), fish (Chen et al., 2015a, 2015b; Jiang et al., 2019), and ducks (Tang et al., 2014; Zhang et al., 2020). Glutathione reductase (GR) is an FAD-dependent enzyme that catalyzes the reduction of glutathione disulfide to reduced glutathione (GSH). Riboflavin deficiency decreases GSH concentrations and GR activity and enhances lipid peroxidation products (Levin et al., 1990; Tang et al., 2014; Chen et al., 2015a; Jiang et al., 2019; Olfat et al., 2022; Zhu et al., 2022). Dietary riboflavin supplementation improved the quality of fish flesh, attributed to increased antioxidant capacity by the Nrf2 signaling pathway (Jiang et al., 2019).

Riboflavin is also involved in lipid metabolism. Fatty acyl-CoA dehydrogenase is an FAD-dependent enzyme that catalyzes the initial rate-limiting step of the fatty acid beta oxidation cycle. Riboflavin deficiency alters lipid metabolism, probably by impairing fatty acid beta oxidation, with changes in liver fatty acid composition and increase incidence of fatty livers (Tang et al., 2017, 2019). In addition, riboflavin status also affects lipid metabolism in muscle. Treatment with riboflavin improved skeletal muscle functions in patients with riboflavin-responsive, multiple acyl-CoA dehydrogenase deficiency (Gianazza et al., 2006). Furthermore, supplementation fish with riboflavin increased muscle unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA), and PUFA (Jiang et al., 2019). However, whether dietary riboflavin supplementation affect breast muscle lipid metabolism of ducks as well as the underlying mechanisms are unknown, which are needed to be investigated.

Therefore, our objectives were to determine effects of dietary riboflavin supplementation on meat quality, antioxidant capacity, fatty acid composition, lipidomic, volatilomic, and proteomic profiling of duck breast muscle.

2. Materials and methods

All experimental procedures were approved by the Animal Welfare Committee of Institute of Animal Science, Chinese Academy of Agricultural Sciences (permission number: IAS2020-41).

2.1. Animals, diets, and experimental design

Female white Pekin ducks (*Anas platyrhynchos*, n = 144, 14-day-old) were sourced from the Chinese Academy of Agricultural Sciences Pekin duck breeding farm. Ducks were randomly allocated to 24 raised plastic-floor pens, each containing six birds. There are three dietary groups, each with eight replicate pens. From 15 to 42 days of age, there was continuous light, air temperature of 20 °C ~ 30 °C and *ad libitum* access to water and feed.

The corn and soybean-based basal diet contained 1.31 mg riboflavin/kg (Table S1). To produce the three experimental diets, basal diets were supplemented with 0, 0.5, or 10 mg /kg diet of riboflavin (purity, 99%; Sigma-Aldrich, St. Louis, MO, USA).

2.2. Data and sample collection

At 42 days of age, following a 12-hour fasting, birds and residual feed from each pen were weighed to calculate average daily weight gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). Then, two ducks from each pen were randomly selected and euthanized by CO_2 inhalation. Subcutaneous fat, breast meat, leg meat, and abdominal fat were isolated and weighed. The left breast muscle was used to assess meat quality, whereas the right breast was snap frozen in liquid nitrogen and stored at −80 °C.

2.3. Riboflavin, FMN, and FAD content

Feed and breast muscle riboflavin, FMN, and FAD contents were measured with reversed-phase high performance liquid chromatography (HPLC), as described (Tang et al., 2014). Pre-treatment of feed and breast muscle samples were done as described (Batey and Eckhert, 1990; Britton et al., 2003). Pure, authentic standards of riboflavin, FMN, and FAD were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Meat quality

Intramuscular fat (IMF) content of duck breast muscle was measured with a Soxtec TM 2050 automated Analyzer (FOSS Analytical, Hillerød, Denmark). Meat pH, color, and drip loss were determined 24 h post mortem. The pH was determined with a pH meter (HI99163; Hanna Instruments Inc., Woonsocket, RI, USA) at three locations in each sample. Lightness (L*), redness (a*), and yellowness (b*) were determined with a chromameter (CR-410, Konica Minolta, Tokyo, Japan) at three locations in each sample. To determine drip loss, ~5 g of breast muscle was weighed, kept in a bag at 4 °C, and reweighed after 24 h.

2.5. Antioxidant status

Breast muscle malondialdehyde (MDA), GSH, and protein carbonyl contents, plus total superoxide dismutase (*T*-SOD), GR, and total antioxidant capacity (*T*-AOC) activities were measured using colorimetric methods and commercial kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, Jiangsu, China).

2.6. Fatty acid profiling

Fatty acid profiles in breast muscle were determined with gas chromatography (7890 A GC-FID system; Agilent Technologies, Palo Alto, USA) as described (Fan et al., 2020). Peaks were identified by corresponding standards (Sigma-Aldrich, St. Louis, MO, USA).

2.7. Lipidomic analysis

Samples of duck breast from the CON and 10 mg/kg riboflavin groups were selected to perform lipidomic analysis. An aliquot (20 ± 1 mg powder) of each breast muscle sample was weighed and homogenized in 1 mL lipid extraction solution (methyl *tert*-butyl ether:methanol, 3:1), then extracted lipids overnight at 4 °C. Then, 200 μL water was added and mixed for 15 min, and then centrifuged 12,000 rpm at 4 °C for 10 min. Thereafter, 300 μL supernatant was removed, concentrated, and stored at −80 °C. The lipidomic analysis were performed with an LC-ESI-MS/MS system (HPLC, Shim-pack UFLC Shimadzu CBM30A system, Shimadzu, Kyoto, Japan; MS, Applied Biosystems 6500 QTRAP, Foster City, CA, USA) equipped with an ESI Turbo Ionspray interface according to the method reported previously (Liu et al., 2023). Orthogonal partial least squares discriminate analysis (OPLS-DA) was done with MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). Any lipid with variable importance in projection (VIP) value >1.0 and $P < 0.05$ was designated as being significantly different between the two groups.

2.8. Volatile compounds analysis

Samples of duck breast from the CON and 10 mg/kg riboflavin groups were used to analyse volatile compounds. Samples were heated in a water bath (80 °C for 30 min), then ground in liquid nitrogen. Breast muscle volatile compounds were determined using a Q Exactive GC Orbitrap-MS (Thermo Fisher Scientific, Bremen, Germany), as described (Li et al., 2022). A volatile compound was considered significantly

different between two groups based on a fold change (FC) > 2 and $P <$ 0.05 (Student's *t*-test).

2.9. Proteomics

Three breast muscle samples from each of the CON group and 10 mg/kg riboflavin group were chosen for isobaric tags for relative and absolute quantification (iTRAQ) assays. Protein extraction, digestion, and iTRAQ assay were done as described (Tang et al., 2019). The database *UniProt_Mallard_8839* was used for protein identification and quantitation. Any protein with FC > 1.5 and $P < 0.05$ was considered differentially expressed between the two groups and pathway enrichment analysis was assessed in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

2.10. Statistical analyses

Comparisons of all three groups used one-way ANONA followed by Duncan's multiple-range test (SAS 9.3; SAS Institute Inc., Cary, NC, USA), whereas comparisons between two groups were done with Student's *t*-test. For all analyses, $P < 0.05$ was considered significant and data were reported as means and their standard errors. Associations between meat quality (pH_{24h}, L*, a*, or b*) and breast muscle lipid molecules (phosphatidylglycerol [PG] or coenzyme Q [CoQ]) were determined by multivariate correlation analyses.

3. Results

3.1. Growth performance and carcass trait

Compared to the CON group, supplementation with 0.5 or 10 mg/kg riboflavin increased ADG and ADFI ($P < 0.01$; Table 1) and decreased FCR ($P = 0.015$; Table 1). Furthermore, dietary riboflavin supplementation increased carcass percentage, all eviscerated ratio, and breast muscle yield ($P < 0.05$; Table 1). However, there were no significant differences among three groups for leg muscle, subcutaneous fat, or abdominal fat yield ($P > 0.05$; Table 1).

3.2. Chemical composition and meat quality

Concentrations of riboflavin, FMN, and FAD in breast muscle increased in association with dietary riboflavin supplementation ($P <$ 0.001; Table 2). Furthermore, riboflavin supplementation increased

Table 1
Effects of dietary riboflavin supplementation on growth performance and carcass traits of Pekin ducks.

Variable	Dietary riboflavin supplementation (mg/kg)			SEM	*P*-value
	0	0.5	10		
ADG, g/d	74.96[b]	85.77[a]	88.78[a]	1.64	<0.001
ADFI, g/d	183.8[b]	200.7[a]	197.1[a]	2.44	0.006
FCR, g/g	2.48[a]	2.35[ab]	2.22[b]	0.049	0.015
Carcass percentage, %	87.87[c]	88.26[b]	88.82[a]	0.093	<0.001
All eviscerated ratio, %	73.01[b]	74.66[a]	74.72[a]	0.27	0.012
Breast muscle, %	10.18[b]	12.68[a]	13.08[a]	0.29	<0.001
Leg muscle, %	12.27	11.62	11.94	0.23	0.515
Subcutaneous fat, %	26.09	25.41	24.99	0.25	0.186
Abdominal fat, %	1.87	1.99	1.78	0.053	0.262

SEM, standard error of the means; ADG, average daily weight gain; ADFI, average daily feed intake; FCR, feed conversion ratio.
The results are represented as mean values with SEM (n = 8).
Carcass percentage = carcass weight × 100%/live body weight.
All eviscerated ratio = all eviscerated weight × 100%/live body weight.
Yield = (breast meat, leg meat, subcutaneous fat, and abdominal fat) × 100%/all eviscerated weight.
[a-c] Within a row, means without a common superscript differed ($P < 0.05$).

Table 2
Effects of dietary riboflavin supplementation on breast muscle riboflavin content, chemical composition, meat quality, and antioxidant status of Pekin ducks.

Variable	Dietary riboflavin supplementation (mg/kg)			SEM	*P*-value
	0	0.5	10		
Riboflavin, ng/g	147.4[c]	227.4[b]	390.7[a]	21.47	<0.001
FMN, ng/g	24.02[c]	34.30[b]	55.18[a]	2.75	<0.001
FAD, ng/g	190[c]	281[b]	465[a]	24.19	<0.001
Moisture, %	76.43	76.30	75.50	0.23	0.232
Crude protein, %	20.64	21.08	21.08	0.14	0.381
IMF, %	1.42[b]	1.78[a]	1.63[a]	0.091	0.041
pH_{24h}	5.65[b]	5.72[a]	5.71[a]	0.008	0.003
Lightness (L*)	48.4[a]	45.5[b]	43.7[b]	0.50	<0.001
Redness (a*)	13.4[b]	13.9[ab]	14.4[a]	0.17	0.036
Yellowness (b*)	7.72[a]	6.57[b]	6.55[b]	0.18	0.007
Drip loss (%)	29.0	28.1	30.2	0.74	0.523
MDA (nmol/mg prot)	0.21[a]	0.10[b]	0.10[b]	0.013	<0.001
T-SOD (U/mg prot)	0.34[c]	0.38[b]	0.39[a]	0.0042	<0.001
T-AOC (U/mg prot)	0.046[c]	0.13[b]	0.19[a]	0.011	<0.001
GR (U/g prot)	11.3[c]	46.8[b]	49.6[a]	2.95	<0.001
GSH (mg/g prot)	6.75[b]	9.01[a]	10.2[a]	0.35	<0.001
Protein carbonyl (nmol/mg prot)	3.16[a]	1.48[b]	0.85[c]	0.17	<0.001

FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; SEM: standard error of the means; IMF: intramuscular fat; MDA: malondialdehyde; *T*-SOD: total superoxide dismutase; *T*-AOC: total antioxidant capacity; GR, glutathione reductase; GSH, reduced glutathione.
The results are represented as mean values with SEM (n = 8).
[a-c] Within a row, means without a common superscript differed ($P < 0.05$).

breast muscle IMF content ($P < 0.001$; Table 2). However, there were no significant differences among three groups in breast muscle moisture or crude protein contents ($P > 0.05$; Table 2).

Both dietary riboflavin supplementation groups increased pH_{24h} and redness (a*) of breast muscle ($P < 0.05$; Table 2), but decreased lightness (L*) and yellowness (b*) of breast muscle ($P < 0.05$; Table 2).

3.3. Oxidative status

Compared to the CON group, dietary riboflavin supplementation increased *T*-SOD, *T*-AOC, GR, and GSH of breast muscle ($P < 0.05$; Table 2) but decreased MDA and protein carbonyl contents ($P < 0.05$; Table 2). Compared to the 0.5 mg/kg group, the 10 mg/kg had greater *T*-SOD, *T*-AOC, and GR ($P < 0.05$; Table 2), but lower protein carbonyl contents ($P < 0.05$; Table 2).

3.4. Breast muscle fatty acids profile

There were no significant differences among the three groups for SFA or MUFA contents of breast muscle ($P > 0.05$; Fig. 1.A). However, compared to the CON group, both supplemented groups had higher total fatty acids (TFA), PUFA, n3-PUFA, and n6-PUFA contents of breast muscle ($P < 0.05$; Fig. 1.A, Fig. 1.D). To be specific, dietary riboflavin supplementation increased C18:2n6, C20:2, C20:3n6, and C22:6n3 (DHA) contents ($P < 0.05$; Fig. 1.D).

3.5. Lipidomic profiles

To explore lipid changes in breast muscle based on IMF content and fatty acid composition, lipidomic profiling was performed to compare the 10 mg/kg supplementation group and the CON group. In total, 688 lipids were identified and analysed. Dietary riboflavin supplementation markedly affected breast muscle lipidomics (Fig. 2A); relative intensity responses of individual classes are shown (Fig. 2E and F). For CON samples, relative response was highest in the TG lipid class (31.18%), followed by PE, PC, FA, SL, and PE-P (25.54%, 13.64%, 7.43%, 4.90%, and 3.78%, respectively), with similar trend for the supplemented

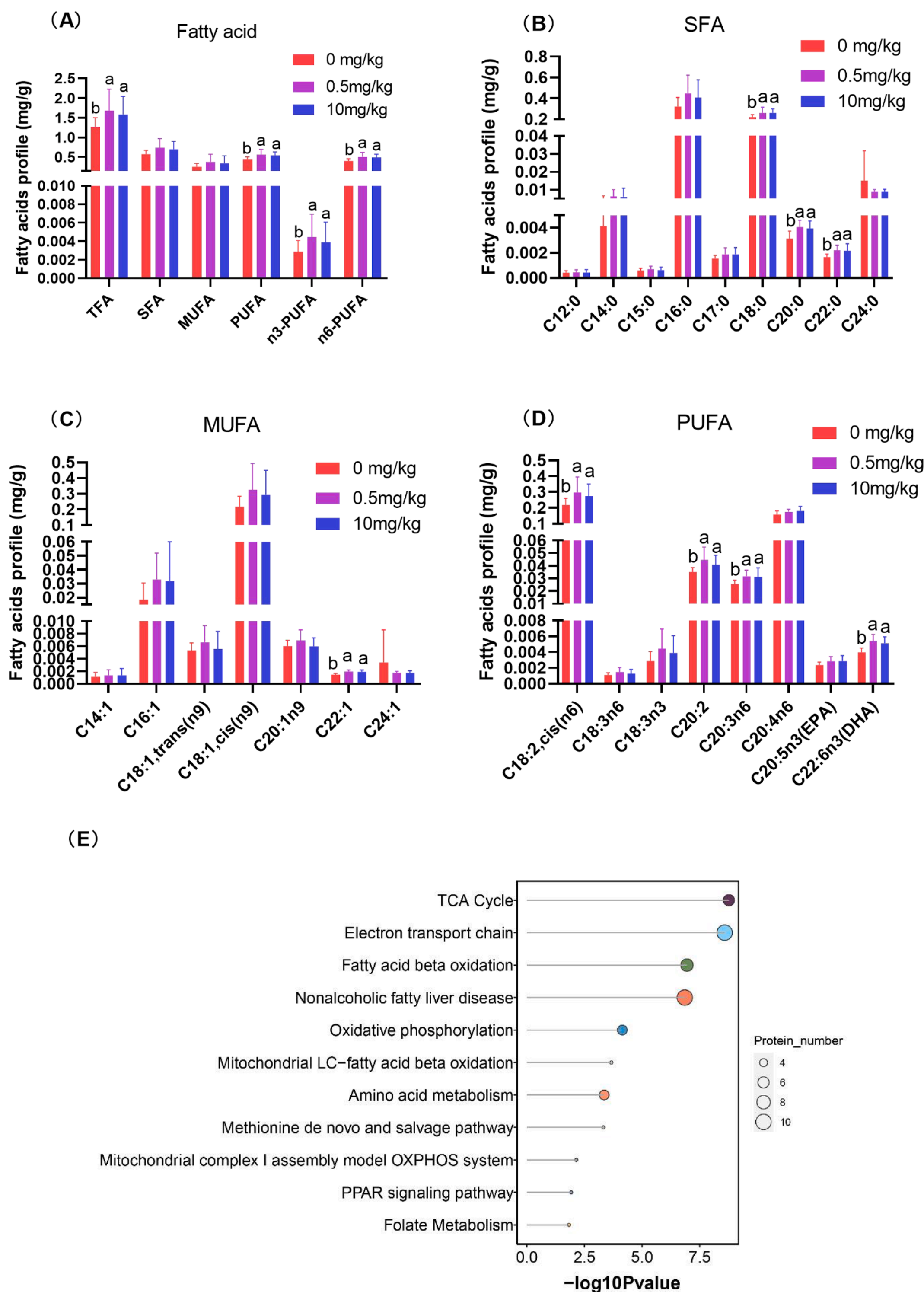

Fig. 1. Effects of dietary riboflavin supplementation on fatty acids composition and protein expression profile of duck breast muscle. (A–D) Effects of dietary riboflavin supplementation on fatty acids profile of duck breast muscle (n = 8). (E) Effects of dietary riboflavin supplementation on protein expression profile of duck breast muscle. Pathway analysis used the Kyoto Encyclopedia of Genes and Genomes (KEGG) to detect differentially expressed proteins in ducks after dietary supplementation with 10 mg/kg riboflavin. [a-b] Bars without a common letter differed ($P < 0.05$). SFA: Saturated fatty acids = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0. MUFA: Monounsaturated fatty acids = C14:1 + C16:1 + C18:1,trans(n9) + C18:1,cis(n9) + C20:1n9 + C22:1 + C24:1. PUFA: polyunsaturated fatty acids = C18:2n6 + C18:3n6 + C18:3n3 + C20:2 + C20:3n6 + C20:4n6 + C20:5 + C22:6. n3-PUFA = C18:3n3 + C20:5n3 (EPA) + C22:6n3 (DHA). n6-PUFA = C18:2n6 + C18:3n6 + C20:3n6 + C20:4n6. TFA: total fatty acids = SFA + MUFA + PUFA.

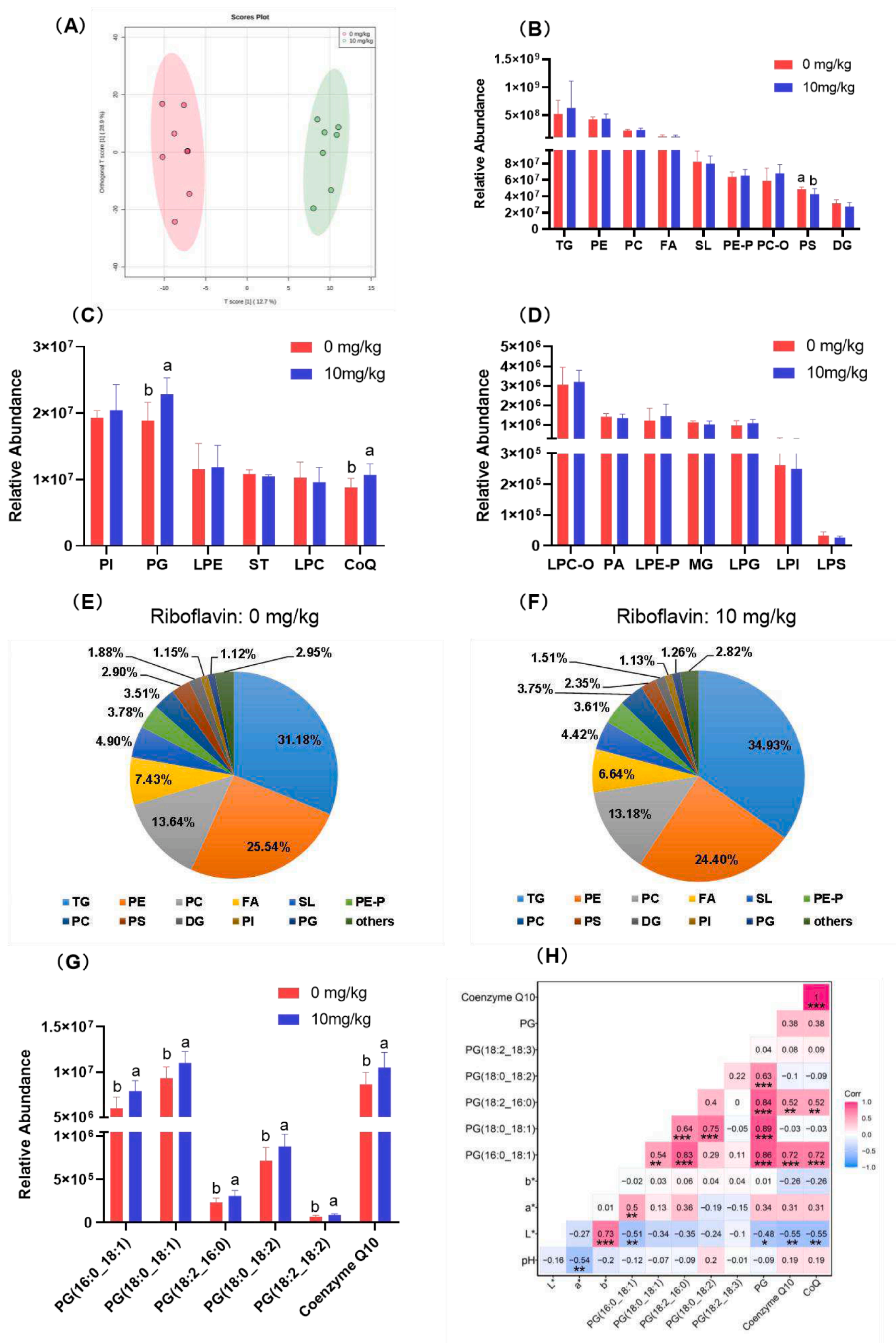

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Fig. 2. Based on lipidomic analysis, lipid profiles of breast muscle in Pekin ducks greatly altered by dietary supplementation with 10 mg/kg riboflavin (n = 8). (A) Orthogonal partial least squares discriminant analysis (OPLS-DA) analysis. (B-D) Comparison of lipid classes between the 10 mg/kg riboflavin supplementation group and the CON group without riboflavin supplementation. (E–F) Relative intensity responses of lipid classes identified in duck breast muscle in the CON group and the riboflavin supplementation group. (G) Representative lipid molecules with VIP scores > 1 in the OPLS–DA and P < 0.05 (Student's t-tests). (H) Correlation coefficients between meat quality (pH_{24h}, L*, a*, or b*) and breast muscle lipid molecules (PG or CoQ). CoQ, coenzyme Q; DG, diglyceride; FA, fatty acids; LPC, lyso-phosphatidylcholine; LPC-O, lyso-plasmanyl-phosphatidylcholine; LPE, lyso-phosphatidylethanolamine; LPE-P, lyso-plasmenyl-phosphatidylethanolamine; LPG, lyso-phosphatidylglycerols; LPI, lyso-phosphatidylinositols; LPS, lyso-phosphatidylserines; MG, monoacylglycerol; PA, phosphatidic acids; PC, phosphatidylcholine; PC-O, plasmanyl-phosphatidylcholine; PE, phosphatidylethanolamine; PE-P, plasmenyl-phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositols; PS, phosphatidylserines; SL, saccharolipids; ST, sterol lipids; TG, triglyceride. CON, control group without riboflavin supplementation. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

group.

In total, 118 lipid molecules differed between the two groups. Compared to the CON group, 33 lipid molecules were upregulated in the riboflavin supplementation group, whereas 85 lipid molecules were downregulated (Table S2). Compared to the CON group, PG and CoQ contents were increased by 20.90% and 21.35%, respectively ($P < 0.05$; Fig. 2C), whereas PS content was decreased by 12.65% ($P < 0.05$; Fig. 2B). Specifically, all differential PG and CoQ were upregulated by dietary riboflavin supplementation, including PG(16:0_18:1), PG(18:0_18:1), PG(18:2_16:0), PG(18:0_18:2), PG(18:2_18:2), and coenzyme Q10 ($P < 0.05$; Fig. 2G). Furthermore, PG(16:0_18:1), PG, coenzyme Q10, or CoQ content were all negatively correlated with the L* value ($P < 0.05$; Fig. 2H), whereas there was a positive correlation between PG(16:0_18:1) and the a* value ($P < 0.05$; Fig. 2H).

3.6. Volatile compounds

A total of 101 volatile compounds were identified and semi-quantified in breast muscle (Table S3). Among these, compared to the CON group, 13 volatile compounds were significantly altered in the 10 mg/kg group, with two volatile compounds increased and 11 volatile compounds decreased. Concentrations of citronellyl acetate and 3-(methylthio)-propanal were 3-fold greater in the riboflavin supplementation group versus the CON group ($P < 0.05$; Table 3). In addition, 11 volatile compounds were significant decreased by dietary riboflavin supplementation ($P < 0.05$; Table 3).

3.7. Proteomics

In total, 1641 proteins were identified by proteomics analysis. Compared to the CON group, 41 proteins were upregulated and 32 were downregulated in the riboflavin supplementation group (Table S4).

Differentially expressed proteins were enriched in the TCA cycle, electron transport chain, fatty acid beta oxidation, nonalcoholic fatty liver disease, oxidative phosphorylation, mitochondrial LC-fatty acid beta oxidation, amino acid metabolism, methionine de novo and salvage pathway, mitochondrial complex I assembly model OXPHOS system, PPAR signaling pathway, and folate metabolism (Fig. 1E). Proteins involved in the energy metabolism are listed in Table 4.

Dietary riboflavin supplementation upregulated seven proteins involved in fatty acid beta oxidation, including short chain acyl-CoA dehydrogenase (ACADS), medium chain specific acyl-CoA dehydrogenase (ACADM), long chain acyl-CoA dehydrogenase (ACADL), long chain fatty acid CoA ligase 6 (ACSL6), dihydrolipoyl dehydrogenase (DLD), glycerol-3-phosphate dehydrogenase 2 (GPD2), and electron transfer flavoprotein alpha subunit (ETFA).

There were five proteins upregulated involved in the TCA cycle by riboflavin supplementation, including DLD, succinate dehydrogenase [ubiquinone] flavoprotein subunit (SDHA), succinate dehydrogenase [ubiquinone] iron-sulfur subunit (SDHB), succinate dehydrogenase cytochrome b560 subunit (SDHC), and succinate dehydrogenase [ubiquinone] cytochrome *b* small subunit (SDHD). In addition, succinate-CoA ligase subunit beta (SUCLA2) was downregulated.

In riboflavin-supplemented ducks, there was upregulation of 10 proteins involved in oxidative phosphorylation, including NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9 (NDUFB9), NADH: ubiquinone oxidoreductase core subunit V2 (NDUFV2), NDUFA4 mitochondrial complex associated (NDUFA4), NADH:ubiquinone oxidoreductase core subunit S1 (NDUFS1), NADH:ubiquinone oxidoreductase core subunit S7 (NDUFS7), SDHA, SDHB, SDHC, SDHD, and ubiquinol-cytochrome *c* reductase core protein 2 (UQCRC2).

Table 3
Effects of dietary riboflavin supplementation on volatile compounds in breast muscle of Pekin ducks.

Compound	RT (min)	Calculated LRI[1]	Literature LRI[2]	Concentration (μg/kg)		SEM	Fold change[3]	P-value[4]
				0 mg/kg	10 mg/kg			
2,4-dimethyl-heptane	5.83	796	797	94.36^{a}	22.27^{b}	61.10	−4.24	0.004
1-decanol	30.85	1654	1764	10.89^{a}	3.08^{b}	8.09	−3.54	0.018
carbon disulfide	5.29	726	730	700.64^{a}	208.55^{b}	333.55	−3.36	0.000
nonanoic acid methyl ester	25.92	1494	1491	2.67^{a}	0.94^{b}	1.52	−2.86	0.005
2-butyl-2-octenal	31.40	1673	1682	48.59^{a}	19.37^{b}	29.96	−2.51	0.017
2-pentadecanone	40.17	1995	2019	3.29^{a}	1.47^{b}	1.64	−2.24	0.007
(E)-2-decenal	30.70	1649	1659	15.09^{a}	7.00^{b}	7.28	−2.16	0.007
(E)-2-undecenal	33.82	1757	1752	11.51^{a}	5.48^{b}	5.57	−2.10	0.008
hexanoic acid methyl ester	15.60	1187	1184	4.19^{a}	2.03^{b}	2.02	−2.06	0.009
1-(1-cyclohexen-1-yl)-1-propanone	29.15	1597	1620	10.71^{a}	5.25^{b}	5.22	−2.04	0.010
(E,Z)-2,4-decadienal	34.18	1770	1754	14.26^{a}	7.06^{b}	8.02	−2.02	0.026
citronellyl acetate	30.43	1640	1660	3.45^{b}	11.01^{a}	8.21	3.19	0.023
3-(methylthio)-propanal	24.82	1460	1454	1.03^{b}	3.58^{a}	2.71	3.47	0.021

RT, retention time; LRI, linear retention index; SEM: standard error of the means.

[1] Linear retention index calculated using the *n*-alkanes (C10-C25) on a DB-Wax capillary column (30 m × 0.25 mm × 0.25 mm) in the gas chromatography mass spectrometry analysis.

[2] Reported linear retention index.

[3] Fold change is expressed as the ratio of the 10 mg/kg riboflavin supplementation group to the control (CON) group without riboflavin supplementation. For downregulated volatile compounds, the fold change was transformed to the corresponding negative value.

[4] Significance of the effect of dietary riboflavin supplementation on the semi-quantitative abundance of each volatile compound based on Student's *t*-test.

Table 4
Effects of dietary riboflavin supplementation on selected differentially expressed proteins in breast muscle of Pekin ducks.

UniProtKB ID	Protein name	Short name	Fold change[1]	*P*-Value
Fatty acid beta oxidation				
U3IAY7	Long chain acyl-CoA dehydrogenase	ACADL	8.39	3.16E−06
U3ITA9	Medium chain specific acyl-CoA dehydrogenase	ACADM	9.28	6.71 E−07
U3J8W0	Short chain acyl-CoA dehydrogenase	ACADS	2.68	2.60 E−03
U3I5L4	Long chain fatty acid CoA ligase 6	ACSL6	2.39	4.56 E−07
U3IR48	Dihydrolipoyl dehydrogenase	DLD	3.01	1.41 E−03
R0K2W7	Glycerol-3-phosphate dehydrogenase 2	GPD2	6.21	2.51 E−06
U3J7F4	Electron transfer flavoprotein alpha subunit	ETFA	3.08	2.02 E−04
TCA cycle				
U3IR48	Dihydrolipoyl dehydrogenase	DLD	3.01	1.41 E−03
U3IHF6	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	SDHA	10.49	5.14 E−06
U3J5X3	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	SDHB	8.23	2.55 E−05
R0KXM3	Succinate dehydrogenase cytochrome b560 subunit, mitochondrial	SDHC	5.47	4.60 E−04
U3IE37	Succinate dehydrogenase [ubiquinone] cytochrome *b* small subunit	SDHD	3.59	9.11 E−09
R0KC12	Succinate-CoA ligase subunit beta	SUCLA2	−3.00	4.71 E−04
Oxidative phosphorylation				
R0JTT3	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 9	NDUFB9	4.63	1.98 E−04
U3IKH0	NADH:ubiquinone oxidoreductase core subunit V2	NDUFV2	2.35	1.20 E−03
U3J9G0	NDUFA4 mitochondrial complex associated	NDUFA4	2.22	1.70 E−06
U3I998	NADH:ubiquinone oxidoreductase core subunit S1	NDUFS1	2.06	6.47 E−05
U3IE92	NADH:ubiquinone oxidoreductase core subunit S7	NDUFS7	1.47	3.04 E−06
U3IHF6	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	SDHA	10.49	5.14 E−06
U3J5X3	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial	SDHB	8.23	2.55 E−05
R0KXM3	Succinate dehydrogenase cytochrome b560 subunit, mitochondrial	SDHC	5.47	4.60 E−04
U3IE37	Succinate dehydrogenase [ubiquinone] cytochrome *b* small subunit	SDHD	3.59	9.11 E−09
U3I342	Ubiquinol-cytochrome *c* reductase core protein 2	UQCRC2	2.14	2.84 E−03

TCA, tricarboxylic acid.
[1] Fold change is expressed as the ratio of the 10 mg/kg riboflavin supplementation group to the control (CON) group without riboflavin supplementation. For downregulated proteins, the fold change was transformed to the corresponding negative value.

4. Discussion

In this study, we explored the effects of dietary riboflavin supplementation on growth performance, meat quality, antioxidant capacity, fatty acid composition, lipidomic, volatilomic, and proteomic profiling of breast muscle in Pekin ducks. There were growth depression, low breast muscle yield, and poor riboflavin status (lower breast riboflavin, FMN, and FAD concentrations) in ducks fed a basal diet without riboflavin supplementation, confirming our previous studies (Tang et al., 2014, 2015, 2017).

Physical indices such as IMF content, pH values, meat color, and water holding capacity are commonly used to evaluate meat quality. Compared to the CON group, dietary supplementation with 10 mg/kg riboflavin improved meat quality, including increased breast IMF content, pH_{24h}, and redness (a*), whereas it decreased lightness (L*) and yellowness (b*). In fish, optimal dietary riboflavin content improved tissue quality (Zehra and Khan, 2018; Jiang et al., 2019), whereas riboflavin deficiency increased the carcass moisture content and decreased crude protein and fat contents (Zehra and Khan, 2018). In another study, riboflavin deficiency reduced muscle moisture, protein, lipid, pH, and shear force in fish, but these were mitigated with adequate dietary riboflavin (Jiang et al., 2019). Perhaps discrepancies between ducks and fish in muscle moisture and protein contents in response to dietary riboflavin supplementation were due to inherent species differences.

Muscle pH is an indicator of the glycolysis rate and lactate accumulation, and directly affects meat color, flavour, and storage period (England et al., 2014). In the present study, dietary riboflavin supplementation significantly increased pH_{24h} of duck breast muscle, indicating a reduced rate of glycolysis and less accumulation. Riboflavin deficiency causes mitochondrial dysfunction and stimulates glycolysis as a compensatory effect (Tang et al., 2017; Cogburn et al., 2018). Furthermore, riboflavin deficiency increased concentrations of muscle lactate in fish (Jiang et al., 2019) and liver lactate in ducks (Tang et al., 2017). In the present study, dietary riboflavin supplementation improved breast muscle color indicated by increased redness (a*) and decreased lightness (L*) and yellowness (b*). Poultry meat with low pH is usually pale (Mir et al., 2017), consistent with our result that breast meat in riboflavin-supplemented ducks had greater pH and was darker. Chinese consumers prefer darker meat (lower L* value) as lighter poultry meat may be interpreted as pale, soft and exudative (PSE) meat.

Compared to the CON group, riboflavin supplementation increased breast muscle concentrations of total fatty acids, PUFA, n3-PUFA (especially DHA), and n6-PUFA. The PUFA, especially omega-3 fatty acids (such as DHA, EPA), have many beneficial nutritional and physiological effects, preventing obesity, cardiovascular, and inflammatory diseases (Zhang et al., 2019a). Riboflavin deficiency alters liver fatty acid composition and causes fatty livers, probably by impairing fatty acid beta oxidation (Tang et al., 2017). Optimal dietary riboflavin supplementation increased muscle UFA, MUFA, and PUFA in fish muscle (Jiang et al., 2019). In skeletal muscle, riboflavin rescues riboflavin-responsive, multiple acyl-CoA dehydrogenase deficient patients (Gianazza et al., 2006).

In the present study, dietary riboflavin supplementation increased *T*-SOD, *T*-AOC, GR, and GSH in breast muscle, and decreased MDA and protein carbonyl contents. Therefore, riboflavin supplementation enhanced breast muscle antioxidant capacity in ducks, suppressing lipid and protein oxidation, consistent with previous studies (Levin et al., 1990; Tang et al., 2014; Chen et al., 2015a; Jiang et al., 2019; Zhang et al., 2020; Zhu et al., 2022). Regarding antioxidant capacity, GR catalyzes generation of GSH from glutathione disulfide, an FAD-dependent enzyme. Riboflavin depletion decreased GR activity and GSH concentration, causing oxidative damage in animals (Levin et al., 1990; Tang et al., 2014; Chen et al., 2015a; Jiang et al., 2019; Zhang et al., 2020; Zhu et al., 2022). Oxidative stress decreases productive performance and meat quality of animals (Falowo et al., 2014; Xing et al., 2019).

However, meat quality is improved by increasing antioxidant capacity by supplementing the diet with antioxidants, such as vitamin C (Skřivan et al., 2012), vitamin E (Bellés et al., 2018), sodium butyrate and vitamin D_3 (Gao et al., 2022), resveratrol (Jin et al., 2021), and riboflavin (Jiang et al., 2019). In the present study, improvements in meat quality were attributed to riboflavin-induced increases in antioxidant capacity. In fish, dietary riboflavin supplementation improved meat quality, partly by activation of the antioxidant system via Nrf2 signaling pathway (Jiang et al., 2019).

To explore the underlying mechanisms of muscle metabolic reprogramming induced by dietary riboflavin supplementation, 10 mg/kg riboflavin group and CON group were selected to perform breast muscle multi-omics, including lipidomic, volatilomic, and proteomic. In the present study, riboflavin supplementation increased breast muscle PG and CoQ contents, such as PG(16:0_18:1), PG(18:0_18:1), PG (18:2_16:0), PG(18:0_18:2), PG(18:2_18:2), and coenzyme Q10, which may have contributed to increased breast IMF content. Phospholipids include many types of UFA, thus contributing to meat flavour (Khan et al., 2015). Therefore, dietary riboflavin supplementation may be beneficial to the enhanced UFA and meat flavour in the present study. PG is a precursor to cardiolipin (CL) and both are constituent molecules of the mitochondrial membrane. Furthermore, PG has essential roles in maintaining mitochondrial membrane structure, respiration, mitophagy, and fusion/fission, whereas PG deficiency causes mitochondrial dysfunction (Kawasaki et al., 1999; Zhang et al., 2019b). In addition, CoQ, primarily in the mitochondrial membrane, is a potent cellular antioxidant, with important roles in regulating mitochondrial functions, including oxidative phosphorylation and fatty acid oxidation (Stefely and Pagliarini, 2017), consistent with the enhanced antioxidant capacity of breast muscle observed in riboflavin supplemented ducks. Interestingly, breast muscle PG or CoQ contents were significantly correlated to meat color (L* value and a* value), with potential as a meat quality biomarker.

Dietary riboflavin supplementation increased concentrations of citronellyl acetate and 3-(methylthio)-propanal >3-fold. Citronellyl acetate is a fresh lemon scent, widely to flavour foods and beverages (Elsharif and Buettner, 2017) whereas 3-(methylthio)-propanal is a baked potato and mushroom flavour that contributes to the aroma of chicken (Fan et al., 2018). Increased concentrations of these two key odorants in duck breast meat by dietary riboflavin supplementation could improve meat flavour.

Based on breast muscle proteomics, riboflavin supplementation mainly upregulated TCA cycle, electron transport chain, and fatty acid beta oxidation. Interestingly, supplementation upregulated all flavoproteins in muscle, including ACADS, ACADM, ACADL, DLD, ETFA, and SDHA, confirming previous findings in duck liver (Tang et al., 2017, 2019). This finding was consistent with the speculation that riboflavin deficiency could reduce flavoprotein expression (Gianazza et al., 2006). Riboflavin has essential roles in maintaining mitochondrial function, and riboflavin deficiency could cause mitochondrial dysfunction (Lee et al., 2013; Tang et al., 2017, 2019). In the present study, dietary riboflavin supplementation upregulated seven proteins involved in fatty acid beta oxidation, five of six proteins involved in the TCA cycle, and 10 proteins involved in oxidative phosphorylation, indicating mitochondrial oxygen respiration was stimulated. These results were consistent with our previous findings that riboflavin deficiency impaired the aforementioned mitochondrial energy generation processes in duck liver (Tang et al., 2017, 2019). Furthermore, riboflavin supplementation presumably activated mitochondria oxygen respiration of breast muscle, associated with higher PG and CoQ concentrations. The CON ducks had poor breast muscle color, perhaps due to mitochondrial dysfunction, which is known to decrease meat quality, e.g., development of PSE-like meat (Liao et al., 2022).

5. Conclusions

Compared to the CON group, 10 mg/kg riboflavin markedly increased growth performance, breast meat yield, breast meat IMF content, PUFA, n3-PUFA, n6-PUFA, redness (a*), pH_{24h}, and antioxidant capacity, and decreased meat lightness (L*) and yellowness (b*). Lipidomic and volatilomic analysis revealed that dietary riboflavin supplementation significantly increased breast meat PG and CoQ concentrations, and two key odorants, citronellyl acetate and 3-(methylthio)-propanal. Furthermore, based on proteomics, dietary riboflavin supplementation stimulated mitochondrial aerobic respiration indicated by enhanced fatty acid beta oxidation, TCA cycle, and oxidative phosphorylation. Taking together, dietary riboflavin supplementation improved the breast meat quality of ducks probably by enhancing antioxidant capacity and mitochondrial function. These findings provided evidence for a practical approach to improve meat quality of ducks, and contributed to our understanding of underlying mechanisms.

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CRediT authorship contribution statement

Jing Tang: Investigation, Formal analysis, Writing – original draft. **Bo Zhang:** Methodology, Validation. **Dapeng Liu:** Data curation. **Kexin Gao:** Investigation. **Ye Dai:** Methodology. **Suyun Liang:** Methodology. **Wentao Cai:** Methodology. **Zhinan Li:** Validation. **Zhanbao Guo:** Investigation. **Jian Hu:** Methodology. **Zhengkui Zhou:** Methodology. **Ming Xie:** Supervision. **Shuisheng Hou:** Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100799.

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