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Dietary salidroside supplementation improves meat quality and antioxidant capacity and regulates lipid metabolism in broilers

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1. Introduction

Consumers have a strong preference for chicken meat due to the many benefits it offers, including high protein content, low levels of fat, and affordable price (Fan et al., 2018). As the demand for chicken continues to rise, the broiler breeding industry has adopted a highdensity breeding approach to achieve rapid gain in the weight of broilers. This intensified farming model has significantly enhanced feed efficiency and production performance in broilers. However, this model makes broilers more susceptible to oxidative stress, leading to various diseases and a decline in meat quality (Julian, 1998; Poompramun et al., 2022). The literature indicates that rapid growth rates adversely affect the pH value, color, and water-holding capacity of broiler chicken muscles (Poompramun et al., 2022). Additionally, myopathies like white striping and woody breast are common in broilers with fast growth rates (Kuttappan, Hargis, & Owens, 2016). Though the use of antibiotics promotes broiler growth and prevents diseases, concerns about antibiotic resistance and drug residues have emerged, which pose a serious threat to food safety (Keulers et al., 2022). Consequently, prohibiting antibiotic use in broiler farming is crucial, and it is urgent to develop novel alternatives to antibiotics that strengthen broiler immunity.

ABSTRACT

We aimed to explore the effect of salidroside (SAL) on meat quality, antioxidant capacity, and lipid metabolism in broilers. The results demonstrated that SAL significantly reduced the yellowness (b^*), shear force, cooking loss, drip loss, MDA, TBARS, and carbonyl content in breast (P < 0.05), while increasing the pH value (P < 0.05), suggesting an improvement in meat quality. SAL lowered the lipid contents in liver and serum (P < 0.05), while increasing the proportion of unsaturated fatty acids in breast (P < 0.05), indicating effective regulation of lipid metabolism by SAL. SAL increased the activity of antioxidant enzymes and the expression of antioxidant genes in both liver and muscle (P < 0.05). Additionally, SAL improved the meat quality and antioxidant capacity of breast subjected to repeated freeze-thaw treatment. SAL may enhance meat quality by improving antioxidative stability and regulating lipid metabolism, potentially serving as a dietary supplement for broilers.

Numerous studies have demonstrated that incorporating natural antioxidants, such as Chinese herbal ingredients, into the diet can increase the antioxidant capacity of broilers and enhance meat quality (Gao et al., 2022; Valentini et al., 2020; Wang et al., 2023).

Metabolic diseases including atherosclerosis, coronary heart disease, and fatty liver are intricately linked to lipid content. Prevention of cardiovascular disease is greatly aided by paying attention to both the total lipid intake and fatty acid ratio in daily diet (Del Gobbo et al., 2016). For instance, the presence of a significant proportion of unsaturated fatty acids (UFA) in chicken breast meat has the potential to decrease the atherosclerosis index (AI) and thrombosis index (TI) (Kumar et al., 2020). Moreover, the fatty acids generated through the degradation of lipid oxidation significantly impact the quality and flavor of meat (Shahidi & Hossain, 2022). Adjusting the nutritional composition of the chicken diet and incorporating supplements are viable strategies to enhance chicken meat quality and flavor. Dietary supplementation of glycerol monolaurate reduced lipid peroxidation levels and proportion of saturated fatty acids (SFA) in broilers (Valentini et al., 2020). Furthermore, supplementing chicken diets with sodium butyrate and/or vitamin D3 could modify oxidative stability, composition of fatty acids, and meat quality (Gao et al., 2022).

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Salidroside (SAL; p-hydroxyphenethyl-β-d-glucoside), derived from the roots of Rhodiola, is a prominent part of traditional Chinese medicine (Gao et al., 2016). SAL is known to have anti-inflammatory, antioxidant, anticancer, immune-enhancing, and lipid-metabolismregulating properties (Zhang et al., 2021). Oral administration of SAL reduced lipid accumulation in mice on the maintenance of a high-fatand cholesterol-containing diet (Hu et al., 2021). Additionally, SAL has been noted for its contributions to osteogenesis and neuroprotection (Sun, Lu, Cai, Zheng, & Zhao, 2020; Xie et al., 2023). In broilers, dietary SAL supplementation could mitigate inflammation, oxidative stress, and lipid-metabolism-related disorders (Cui et al., 2022). SAL could considerably alleviate oxidative damage and exhibited robust antioxidant activity in various tissues and cells (Chen, Zhang, Cheng, & Ding, 2009; Cui et al., 2022). Bioactive compounds with antioxidant properties and regulatory effects on lipid metabolism play significant roles in enhancing meat quality (Cheng et al., 2020; Wang et al., 2020). Therefore, we speculated that SAL might enhance the quality of broiler breast meat, particularly in high-density farming settings. Plant extracts offer plentiful sources, low toxicity, and the absence of drug resistance, rendering them promising clinical prospects for improving the meat quality of broilers. However, few studies have examined how SAL affects the chicken meat quality.

Thus, the objective of our study was to comprehensively evaluate the influence of SAL on breast meat quality and lipid metabolism, oxidation status, antioxidant enzyme activity, fatty acid profile, and chemical composition of chicken meat. Furthermore, we explored the potential correlations among oxidation status, meat quality parameters, and fatty acid profile, offering a theoretical foundation for incorporating SAL supplementation in broiler farming practices.

2. Materials and methods

2.1. Animals and study design

All protocols and broilers involved in this experiment received approval from the Nanjing Agricultural University Institutional Animal Care and Use Committee (Permit No: PAT2023050). A total of 96 Arbour Acres broilers, aged 1 day, were obtained from the Hai'an Shuangli poultry hatchery.

The chemical structural formula of SAL and experimental design are presented in Fig. 1 (A and B). The broilers were randomly divided into 4 dietary groups: control (CON), low-dose SAL (LSAL), medium-dose SAL (MSAL), and high-dose SAL (HSAL), receiving a mashed diet supplemented with SAL at different concentrations (0, 200, 400, and 600 mg/ kg of diet, respectively). The feeding period lasted for 42 days. Throughout the experimental duration, the birds could freely obtain sufficient feed and water. SAL was procured from Daosifu Biotechnology Co., Ltd. (Nanjing, China).

After completing the feeding experiment, 8 birds from each group with a weight around average body weight were chosen. After electric shock, blood samples were collected from the pterygoid veins of chickens, and subsequently bled to death. The liver and breast muscle samples were immediately collected. The weights of the spleen, heart, and bursa of Fabricius were assessed. A segment of the left breast muscle was promptly subjected to quick freezing at a temperature of -80 °C for subsequent oxidative status analysis and RNA extraction, and the remaining portion was temporarily stored at 4 °C to evaluate fresh meat quality. The samples from the right breast were frozen at a temperature of -20 °C in order to facilitate examination of their chemical composition. These samples were subjected to multiple freeze-thaw cycles in preparation for later investigations. The liver was fixed in formaldehyde, followed by tissue histopathology staining. Fresh liver samples were obtained and promptly stored at -80 °C for subsequent assessment of lipid metabolism parameters and antioxidant enzyme activity.

2.2. Evaluation of meat quality

The chromameter (CR-410, Konica Minolta, Tokyo, Japan) was employed to evaluate the color attributes of the chicken meat, including lightness (L^*), redness (a^*), and yellowness (b^*). The instrument is equipped with a pulsed xenon lamp as the light source, a vertical measurement receiving probe, and a 50 mm aperture. All samples were handled by the same experimenter who was unaware of the experimental grouping. The pH of chicken breast muscle (stored at 4 °C) was evaluated using the HI9125 pH meter (Hanna Instruments, Cluj-Napoca, Romania) at the 45-min and 24-h time points after slaughtering. The color and pH of each sample were tested five times to calculate the average value.

Fresh chicken breast muscle was cut into small pieces (weighing approximately 10 g), and their weights were labeled as *W*1. After being hung at 4 °C for 24 h, their weights were labeled *W*2 after excess surface moisture was blotted from the breast muscle. Drip loss was calculated as per the formula provided in supplementary materials.

The meat samples weighing approximately 30 g (W3) were cut into specific shapes (2 cm \times 2 cm \times 5 cm), and subsequently placed in a plastic bag. These samples were cooked in a thermostat water bath (85 °C) until the meat's internal temperature reached 77 °C. After absorbing the remaining moisture, the weights of the samples were measured and labeled as *W4*. The formula for calculating cooking loss is included in the supplementary materials.

2.3. Chemical composition of meat

The analysis of moisture, ash, crude protein, and crude fat contents in chicken breast flesh was conducted using the recognized procedures outlined by the Association of Analytical Chemists (AOAC, 2000). The levels of moisture were assessed via oven-drying at 110 °C. Kjeldahl technique was used to calculate the amount of crude protein. The Soxhlet method was used for assessing crude fat. The determination of ash content was conducted by measuring the weight of the muscle samples following their exposure to a muffle furnace at a temperature of 550 °C for a duration of 4 h.

2.4. Repeated freeze-thaw of chicken breast muscle

One cycle of freeze–thaw included placing freshly collected right chicken breast muscle at -20 °C for 1 week, followed by thawing and incubating at 4 °C for 24 h. In total, 5 freeze–thaw cycles were performed. After the final thawing cycle, approximately 1 g of the breast muscle was flash-frozen in liquid nitrogen, ground into powder, and dissolved in the specific extraction solution for subsequent analysis of oxidation status. The remaining chicken breast meat that underwent repeated freeze–thaw cycles was used to assess the meat color, drip loss, cooking loss, pH, and shear force.

2.5. Measurement of lipid content

Using respective commercial kits as per the manufacturer's instructions, the levels of triglycerides (TG), total cholesterol (TC), lowdensity lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL—C) in the serum and liver were measured (Nanjing Jiancheng Biotechnology Co., Ltd., Nanjing, China).

2.6. Determination of antioxidation and lipid oxidation status

The activities of antioxidant enzymes, including total superoxide dismutase (T-SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), were measured in both serum and liver samples. The assessment was conducted using commercially available kits provided by Nanjing Jiancheng Biotechnology Co., Ltd., following the manufacturer's instructions. The oxidation state of both fresh and repeatedly frozen and



Fig. 1. The effect of dietary SAL addition on body weight and organ index at broilers. n = 8 per group. (A) Chemical structure of SAL. (B) Schematic diagram showing the study design in broilers. Effect of SAL on body weights (C), spleen muscle index (D), bursa of fabricius index (E), and heart index (F), breast muscle index (G), thigh muscl index (H), liver index (I) of broilers. The organ index was expressed as the ratio of organ weight to body weight. All data are presented as the mean \pm SEM. Various letters indicate statistically significant differences (P < 0.05).

thawed chicken breast meat samples was obtained through assessing total antioxidant capacity (T-AOC) and contents of 1-diphenyl-2-picrylhydrazyl (DPPH), malondialdehyde (MDA), non-esterified fatty acids (NEFA), and carbonyl the appropriate diagnostic kits provided by Nanjing Jiancheng Biotechnology Co., Ltd. The TBARS level was expressed as mg MDA/kg meat (Wang et al., 2023).

2.7. Real-time PCR

The liver and muscle tissues were rapidly frozen in liquid nitrogen and then finely ground to form powder. Total RNA was extracted using Trizol (Angle Gene Biotechnology Co. Ltd., Nanjing, China). RNA concentration was determined using the NanoDrop 2000 Spectrophotometer. The RNA was reverse-transcribed using a kit to form cDNA (TransGen Biotech Biotechnology Co. Ltd., Nanjing, China). The transcript levels of genes of interest, specifically those associated to lipid metabolism and antioxidant activity, were analyzed using real-time PCR. Table S1 contains the primer sequences. The quantification of the transcript levels of target genes was performed using the $2^{-\Delta\Delta CT}$ method, with *GAPDH* serving as the reference.

2.8. Histopathology

Liver samples were fixed in paraformaldehyde, dehydrated using ethanol gradient, decolorized using xylene, embedded in paraffin, and sectioned. Subsequently, hematoxylin and eosin (H&E) were used to stain the sections to visualize the liver structure (Wan et al., 2021). Additionally, sections of frozen liver samples were subjected to staining with Oil Red O to evaluate the quantity and dimensions of lipid droplets (Pinuofei Services Biotechnology Co., Ltd., Wuhan, China).

2.9. Fatty acid profile of broiler meat

In total, 0.1 g of the samples was extracted in chloroform and dichloromethane mixture. Fatty acid profiles were analyzed using the Agilent gas chromatography system (Agilent 7820; Agilent Technologies, USA) with the CP-Sil 88 gas chromatographic column (100 m \times 0.25 mm \times 0.25 µm, Agilent, USA) selected based on the properties of different compounds, 1 µL injection volume, 10:1 split ratio, and highpurity helium as the carrier gas at a flow rate of 1.0 mL/min. Initially, the temperature of the column was maintained at 100 °C for 5.0 min. Then, it was gradually increased to 240 °C at 4 °C/min for 15 min. Subsequently, a mass spectrometry system (Agilent 5977; Agilent Technologies, USA) was used to identify and quantify the fatty acids. Data were acquired using MassHunter GC/MS Acquisition (Agilent Technologies). The detection of fatty acid composition in broilers was completed by Sanshu Biotechnology Co., Ltd.

2.10. Health indices of meat

Total fat, saturated fatty acids (SFA), UFA, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), n-3 PUFA, n-6 PUFA, SFA/UFA ratio, and n-6/n-3 PUFA ratio were evaluated based on the fatty acid profile. The AI and TI were determined using methods described previously (Ulbricht & Southgate, 1991). Additionally, hypercholesterolemic fatty acids (HFA), desirable fatty acids (DFA), nutritive value index (NVI), and health-promoting index (HPI) of broiler muscle were assessed as per the formula provided in supplementary materials (Akinbule, Onabanjo, Sanni, Adegunwa, & Akinbule, 2022; Kumar et al., 2020).

2.11. Statistical analysis

Using the Shapiro–Wilk test, all data were assessed for normality. One-way ANOVA (for multiple groups) and student's *t*-test (for two groups) were employed for comparisons (SPSS 26.0). P < 0.05 was considered statistically significant. Pearson's test was employed for correlation analyses. Graphs were generated using GraphPad Prism 8.0. Fatty acid profile data were processed using Quant-My-Way (Agilent Technologies). All data were expressed as the mean \pm standard error of the mean (SEM).

3. Results

3.1. Body weight and organ index

All doses of SAL did not significantly alter the body weight, spleen index, heart index, and bursa of fabricius index of the broilers (P > 0.05, Fig. 1 C—F). Dietary supplementation of SAL improved the breast muscle index of chicken (P < 0.05, Fig. 1 G); however, it was not significantly different among the LSAL, MSAL, and HSAL groups (P > 0.05). The thigh muscle index was higher in the MSAL and HSAL groups than in the CON group (P < 0.05, Fig. 1 H). Notably, the liver index was only lower in the HSAL group than in the CON group (P < 0.05, Fig. 1 I).

3.2. Meat quality of fresh breast muscle

The overall morphological features of the pectoral muscle are shown in Fig. 2 A. Various doses of dietary SAL did not significantly affect the lightness and redness of the breast meat (P > 0.05). However, yellowness of the breast meat significantly decreased in the HSAL group (P < 0.05, Fig. 2 B1-B3). Notably, SAL supplementation significantly reduced the shear force (P < 0.05, Fig. 2 C) and increased the pH value of chicken breast muscle at 45 min and 24 h post-slaughter (P < 0.05, Fig. 2 D1-D2). Furthermore, SAL treatment significantly reduced the cooking loss and drip loss as well (P < 0.05, Fig. 2 E1-E2). Interestingly, the positive impacts of SAL on the shear force, pH value, and water-holding capacity exhibited no significant differences among the LSAL, MSAL, and HSAL groups (P > 0.05).

3.3. Lipid metabolism indicators in the serum and liver

The impact of dietary SAL on the lipid metabolism of broilers is shown in Fig. 3. The SAL supplementation significantly reduced TG, TC, and LDL-C levels while increasing HDL-C level in the liver and serum of broilers (P < 0.05, Fig. 3 A1-A4 and B1-B4). Notably, both H&E and Oil Red O staining demonstrated a reduction in lipid droplets in broiler liver tissue under SAL supplementation. Oil Red O area analysis revealed a decrease in lipid accumulation under SAL supplementation (P < 0.05, Fig. 3 C). The results of qPCR indicated that SAL significantly downregulated the genes linked to lipid metabolism in the liver and breast muscle, including acyl-CoA desaturase 1 (*Scd1*), fatty acid synthase (*Fasn*), acetyl-CoA carboxylase alpha (*Acaca*), and peroxisome proliferator-activated receptor gamma (*Pparg*) (P < 0.05, Fig. 3 D and E). The lipid content decreased with SAL supplementation in a concentration-dependent manner.

3.4. Activity of antioxidant enzymes in the serum and liver

Figure 4 A1-A3 and B1-B3 indicate the antioxidant enzyme activities in the liver and serum of broilers. The HSAL group exhibited significantly higher antioxidant enzyme activity (SOD, CAT, and GSH-Px) compared to the CON group (P < 0.05). The HSAL group exhibited a more prominent antioxidant effect than the LSAL and MSAL groups. Consistent with these results in the serum, various doses of SAL enhanced antioxidant enzyme activity in the liver (P < 0.05), with the HSAL group exhibiting much higher SOD, CAT, and GSH-Px activities than the LSAL and MSAL groups.



Fig. 2. Effect of dietary salidroside (SAL) on meat quality of chicken breast muscle. n = 8 per group. (A) Representative morphological images of breast muscle. (B1-B3) The color [lightness (L^*), redness (a^*), and yellowness (b^*)] of breast muscle. (C) Comparison of shear force. (D1-D2) Comparison of pH value (45 min and 24 h). (E1-E2) Cooking loss and drip loss. All data are presented as the mean \pm SEM. Various letters indicate statistically significant differences (P < 0.05).

3.5. Lipid oxidation parameters in the liver and muscle

As shown in Fig. 4 C1-C2, the lipid oxidation parameters (MDA and TBARS levels) of the liver were reduced by SAL supplementation (P < 0.05) but were comparable among the LSAL, MSAL, and HSAL groups (P > 0.05). The HSAL group exhibited significantly reduced levels of MDA, TBARS, and carbonyl (P < 0.05) and significantly increased contents of DPPH in the breast meat (P < 0.05, Fig. 4 D2-D4 and D6). The MDA and TBARS contents were reduced in the MSAL group in comparison to the CON group (P < 0.05), whereas only the MDA content exhibited a decrease in the LSAL group (P < 0.05, Fig. 4 D2-D3). Surprisingly, no significant difference was observed in T-AOC and NEFA contents among the CON and SAL groups (P > 0.05, Fig. 4 D1 and D5). SAL significantly upregulated the antioxidant-related genes in the liver and breast muscle (P < 0.05), including heme oxygenase 1 (*HO-1*), glutathione (*GSH*), nuclear factor erythroid 2-related factor 2 (*Nrf2*), and *CAT*. Moreover,

the HSAL group exhibited a significant upregulation of antioxidant-related genes when compared with the LSAL or MSAL groups (P < 0.05, Fig. 4 E and F).

3.6. Meat quality and antioxidant activity of breast muscle after repeated freezing and thawing

After repeated freeze–thaw cycles, the impact of SAL on meat quality resembled that of fresh meat. The brightness of chicken breast meat appeared to be similar among all the groups (P > 0.05, Fig. 5 A1). The MSAL and HSAL groups exhibited a reduction in the yellowness of the meat (P < 0.05). Interestingly, the redness of the chicken (P < 0.01) was significantly increased in the HSAL group, which was not observed in fresh chicken breast meat (Fig. 5 A2-A3). In terms of meat acidity after freezing and thawing, the pH value was sequentially higher in the LSAL, MSAL, and HSAL groups than that in the CON group (P < 0.05, Fig. 5 B).



Fig. 3. Effect of dietary SAL addition on lipid metabolism in serum and liver of broilers. (A1-A4)Serum lipid content (TG, TC, LDL-C, and HDL-C). n = 8 per group. (B1-B4) Hepatic lipid content (TG, TC, LDL-C, and HDL-C). n = 8 per group. (C) H&E and Oil Red O staining of liver sections. Scale bar, 200 µm. n = 6 per group. (D-E) Relative mRNA levels of lipid metabolism-related genes (*Scd1*, *Fasn*, *Pparg*, and *Acaca*) in broiler liver (D) and breast muscle (E). n = 8 per group. All data are presented as the mean \pm SEM. Various letters indicate statistically significant differences (P < 0.05). TG: triglyceride; TC: total cholesterol; LDL-C: low-density lipoprotein-cholesterol; HDL-C: high-density lipoprotein cholesterol; *Scd1*: acyl-CoA desaturase 1; *Fasn*: fatty acid synthase; *Pparg*: peroxisome proliferator-activated receptor gamma; *Acaca*: Acetyl-CoA carboxylase alpha. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



Fig. 4. Effect of dietary SAL addition on antioxidant capacity of broilers. (A1-A3) Effect of SAL on serum antioxidant enzyme activities (SOD, CAT, GSH-Px). (B1-B3) Effect of SAL on liver antioxidant enzyme activities (SOD, CAT, GSH-Px). (C1-C2) Effect of SAL on liver lipid oxidation state (MDA and TBARS). (D1-D6) Effect of SAL on breast redox status (T-AOC, MDA, TBARS, DPPH, NEFA, and carbonyl). (*E*-F) Relative mRNA levels of antioxidant-related genes (*HO-1, GSH, CAT,* and *Nrf2*) in broiler liver (E) and breast muscle (F). n = 8 per group. All data are presented as the mean \pm SEM. Various letters indicate statistically significant differences (*P* < 0.05). SOD: superoxide dismutase; CAT: catalase; GSH-Px: glutathione peroxidase; MDA: malondialdehyde; TBARS: Thiobarbituric acid reactive substances; T-AOC: total antioxidant capacity; DPPH: 1-diphenyl-2-picrylhydrazyl; NEFA: non-esterified fatty acids; *HO-1*: heme oxygenase 1; *Nrf2*: nuclear factor erythroid 2-related factor 2.



Fig. 5. The positive effects of SAL on the meat quality and oxidative stability of repeatedly frozen and thawed chicken breast meat (A1-A3) The color [lightness (L^*), redness (a^*), and yellowness (b^*)] of repeated freezing and thawing of the breast muscle. (B) The pH value of repeated freezing and thawing of the breast muscle. (C1-C2) The cooking loss and drip loss of repeated freezing and thawing of the breast muscle. (D) The shear force of repeated freezing and thawing of the breast muscle. (E-J) The redox status (T-AOC, MDA, TBARS, NEFA, DPPH, and carbonyl) of repeated freezing and thawing of the breast muscle. (P < 0.05).

Additionally, cooking loss, drip loss, and shear force were lower in the LSAL, MSAL, and HSAL groups than in the CON group (P < 0.05), with the HSAL group exhibiting the most significant improvement in these parameters (Fig. 5 C1-C2 and D).

The antioxidant effect of SAL in chicken breast meat remained evident despite repeated freeze–thaw cycles. The MSAL and HSAL groups exhibited increased T-AOC content in the breast muscle tissue when compared with the CON group (P < 0.05). In contrast, no significant effect was discovered in the LSAL group (P > 0.05, Fig. 5 E). As

expected, SAL supplementation at all doses reduced the levels of MDA, TBARS, and NEFA (P < 0.05, Fig. 5 F—H). The DPPH content in chicken breast meat was elevated in the LSAL and HSAL groups compared to the CON group (P < 0.05, Fig. 5 I). Only the HSAL group demonstrated a significant reduction in the carbonyl content when compared with the CON group (P < 0.05, Fig. 5 J).

3.7. Chemical composition of the muscle

Supplementation with a high dose of SAL improved the meat quality and antioxidant capacity to the highest extent. Consequently, the differences in the chemical composition and fatty acid profiles of broiler breast muscle were compared between the CON and HSAL groups. SAL supplementation had no impact on the moisture, ash, and crude protein contents in chicken breast meat (P > 0.05, Fig. S1 A, B and D). However, a notable reduction in the concentration of crude fat was observed in the HSAL group in comparison to the CON group (P < 0.05, Fig. S1 C).

3.8. Fatty acid profile and health index analysis of muscle

The fatty acid profiles of chicken breast muscle in the CON and HSAL groups are presented in Table 1. SAL supplementation reduced the content of SFA (P < 0.05), including C12:0, C14:0, C16:0, C18:0, C20:0, and C22:0. The HSAL group exhibited significantly higher content of PUFA (C18:2n6c, C18:3n3, C18:3n6, and C20:3n) than the CON group (P < 0.05). Though the total MUFA content in broiler breast muscle appeared to increase in the HSAL group compared to the CON group, this difference was not statistically significant (P > 0.05). The SFA to UFA ratio decreased after HSAL supplementation (P < 0.05). Additionally, HSAL significantly increased the content of n-3 PUFA and n-6 PUFA and reduced the ratio of n-6 to n-3 PUFA in chicken breast meat (*P*)

Table 1

Effect of SAL on chicken breast muscle's fatty acid profile and health index (n = 6).

Fatty acid (g/100 g)	CON	HSAL	Δ (%)	SEM	P value
C12:0	0.009 ^a	0.008 ^b	-11.11	0.0002	0.001
C14:0	0.070^{a}	0.056 ^b	-20.00	0.0026	0.002
C16:0	2.243 ^a	1.874^{b}	-16.45	0.0674	0.001
C16:1	0.049^{b}	0.121^{a}	146.94	0.0127	< 0.001
C18:0	2.711 ^a	2.306^{b}	-14.94	0.0685	< 0.001
C18:1n9c	1.151	1.330	15.55	0.0652	0.183
C18:2n6c	0.873^{b}	1.256 ^a	43.87	0.0795	0.006
C18:3n3	0.019^{b}	0.037 ^a	94.74	0.0033	0.002
C18:3n6	0.007^{b}	0.009 ^a	28.57	0.0006	0.012
C20:0	0.027^{a}	0.021^{b}	-22.22	0.0011	< 0.001
C20:1	0.011^{b}	0.016 ^a	45.45	0.0009	0.010
C20:2n6	0.037	0.042	13.51	0.0021	0.199
C20:3n3	0.004 ^b	0.005 ^a	25.00	0.0002	0.02
C20:3n6	0.055 ^a	0.042^{b}	-23.64	0.0031	0.02
C20:4n6	0.378	0.343	-9.26	0.0116	0.128
C20:5n3	0.001	0.001	0.00	0.0001	0.515
C22:0	0.015^{a}	0.010^{b}	-33.33	0.0009	0.005
C22:2	0.004	0.006	50.00	0.0006	0.073
C22:6n3	0.005	0.005	0.00	0.0005	0.588
C24:0	0.001	0.001	0.00	0.0001	0.130
Total fat	7.672	7.496	-2.29	0.1330	0.537
SFA	5.076 ^a	4.276 ^b	-15.76	0.1394	< 0.001
MUFA	1.212	1.466	20.96	0.0737	0.083
PUFA	1.384 ^b	1.754 ^a	26.73	0.0768	0.007
n-3 PUFA	0.029^{b}	0.047 ^a	62.07	0.0034	0.003
n-6PUFA	1.314 ^b	1.659 ^a	26.26	0.0726	0.009
n-6: n-3 PUFA	44.598 ^a	35.280^{b}	-20.89	0.0013	0.011
SFA: UFA	1.956 ^a	1.328^{b}	-32.11	0.1028	< 0.001
AI	0.975 ^a	0.654 ^b	-32,92	0.0528	< 0.001
TI	3.658^{a}	2.446^{b}	-33.13	0.2004	< 0.001
DFA	5.307	5.527	4.15	0.1116	0.348
HFA	2.322^{a}	1.938^{b}	-16.54	0.0698	0.001
NVI	1.722^{b}	1.941 ^a	12.72	0.0446	0.005
HPI	1.025^{b}	1.529 ^a	49.17	0.0879	< 0.001

Mean values with different superscript letters within the same row are significantly different (P < 0.05). Δ :%difference of the mean values ([HSAL - CON] / CON).

SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; UFA: Unsaturated fatty acid; AI: Atherogenic index; TI: Thrombogenic index; DFA: Desirable fatty acid; HFA: Hypercholesterolemic fatty acids; NVI: Nutritive value index; HPI: Health promoting index; SEM: Standard error of mean; CON: Control; HSAL: High dose salidroside. < 0.05).

The AI, TI, and HFA values were reduced in the HSAL group relative to the values observed in the CON group (P < 0.05). DFA levels appeared to be comparable between the CON and HSAL groups (P > 0.05). Furthermore, HSAL supplementation increased the NVI and HPI values (P < 0.05).

3.9. Correlation analysis of antioxidant level, fatty acids, and meat quality

To explore the potential mechanisms underlying the enhancement in meat quality under SAL supplementation, Pearson's correlation analysis was performed and the relationships among fatty acid composition, antioxidant indicators, and meat quality parameters were examined. Fig. 6 A indicates a robust correlation between meat quality and antioxidant indicators in chicken breast muscle. Shear force, drip loss, and cooking loss exhibited a significant positive correlation with MDA levels $(0.90 \le r \le 0.91, P < 0.01)$ and a significant negative correlation with DPPH level ($-0.75 \le r \le -0.72$, *P* < 0.01). Moreover, the pH values (45 min and 24 h) were negatively correlated with the MDA level (r = -0.89and -0.90, P < 0.01) and positively correlated with the DPPH level (r =0.75 and 0.76, P < 0.01). Similarly, carbonyl content exhibited a positive correlation with shear force, drip loss, and cooking loss ($0.60 \le r \le$ 0.74, P < 0.05) and a negative correlation with pH values (45 min and 24 h) (r = -0.65 and -0.74, P < 0.01). The transcript levels of antioxidant-related genes were positively correlated with pH values (45 min and 24 h) (0.78 < r < 0.93, P < 0.01) and negatively correlated with shear force, drip loss, and cooking loss ($-0.94 \le r \le -0.79$, $P \le 0.01$).

The *a** value mostly exhibited no significant correlation with antioxidant- and lipid-metabolism-related indicators. Conversely, the *b** value was positively correlated with MDA and carbonyl contents and transcript level of lipid-metabolism-related genes (including *Scd1*, *Fasn*, and *Pparg*) (0.39 $\leq r \leq 0.74$, *P* < 0.05); it exhibited a negative correlation with that of antioxidant genes ($-0.57 \leq r \leq -0.50$, P < 0.05). Therefore, it was evident that SAL supplementation was related to improving chicken meat quality and enhancing antioxidation levels.

A strong correlation was observed between fatty acid composition and meat quality (Fig. 6 A). For instance, crude fat and SFA contents were positively correlated with shear force, drip loss, and cooking loss $(0.67 \le r \le 0.97, P < 0.05)$; they were negatively correlated with pH values $(-0.94 \le r \le -0.8, P < 0.05)$. PUFA, n-3 PUFA, and n-6 PUFA were negatively correlated with shear force, drip loss, and cooking loss $(-0.78 \le r \le -0.59, P < 0.05)$ and positively correlated with pH values $(0.65 \le r \le 0.8, P < 0.05)$. Additionally, AI and TI values and the transcript level of lipid-metabolism-related genes were significantly positively correlated with shear force, drip loss, and cooking loss $(0.71 \le r \le 0.91, P < 0.01)$ and significantly negatively correlated with pH values $(-0.93 \le r \le -0.74, P < 0.01)$. These results indicated that an interaction existed between the meat quality and fatty acid metabolism of chicken breast meat.

Furthermore, the potential relationship between antioxidant capacity and lipid metabolism indicators was examined (Fig. 6 B). The correlation of crude fat and SFA contents with MDA and carbonyl contents was positive ($0.58 \le r \le 0.93$, P < 0.05) and with DPPH content, it was negative (r = -0.74 and - 0.64, P < 0.05). The correlation between MUFA and antioxidant-related parameters was less pronounced. The transcript levels of antioxidant-related genes were significantly negatively correlated with crude fat and SFA contents and AI and TI values ($-0.96 \le r \le -0.74$, P < 0.01). Notably, *Nrf2* exhibited a significant positive correlation with PUFA, n-3 PUFA, and n-6 PUFA ($0.73 \le r \le 0.80$, P < 0.01). Additionally, PUFA, n-3 PUFA, and n-6 PUFA exhibited a positive correlation with T-AOC ($0.68 \le r \le 0.64$, P < 0.05) and a negative correlation with MDA ($-0.64 \le r \le -0.60$, P < 0.05). These findings suggested that the fatty acid composition could influence the antioxidant levels in chicken breast meat.



Fig. 6. Pearson correlation analysis of antioxidant level, fatty acids, and meat quality of chicken breast muscle after dietary SAL supplementation. (A) Correlation heat map between antioxidant-related indexes and fatty acid composition with meat quality. (B) Correlation heat map between antioxidant indices and fatty acid composition. * (P < 0.05) and ** (P < 0.01) indicate statistically significant correlations between the two variables.

4. Discussion

The essential physical parameters for assessing the quality of chicken meat are meat color, drip loss, cooking loss, pH value, and shear force (Mir, Rafiq, Kumar, Singh, & Shukla, 2017). Meat color, a direct indicator of quality, significantly influences the sales and economic value of chicken breast meat (Kennedy, Stewart-Knox, Mitchell, & Thurnham, 2005). Regional preferences vary regarding meat color, with Chinese consumers favoring a reddish hue. In this study, the addition of SAL to the chicken diet notably reduced the yellowness (b*) of chicken breast meat while enhancing its redness (a^*) to a certain extent. The a^* value is primarily determined by the contents of myoglobin and hemoglobin in the muscle and is influenced by both light reflection and oxidation (Turgut, Isikci, & Soyer, 2017). Chicken breast meat contains lower amount of myoglobin, which may be one of the reasons why the a* value did not alter significantly after SAL supplementation. pH serves as a pivotal indicator of meat quality. After slaughtering, lactate accumulation due to anaerobic glycolysis accelerates the decrease in the pH value of meat (Scheffler, Matarneh, England, & Gerrard, 2015). This decline in pH directly impacts the color, shear force, and water retention ability of the muscle. Altering the glycolysis rate and reducing lactate accumulation in muscle can enhance pork quality (Wang et al., 2020). In our study, we observed a gradual increase in the pH of chicken meat at 45 min and 24 h following SAL supplementation. Moreover, after SAL supplementation, chicken breast meat exhibited a significant decrease in shear force, which can be attributed to the increased water retention capacity. Multiple studies demonstrated a positive correlation between shear force and drip loss (Gao et al., 2022; Valentini et al., 2020). Our data indicated that SAL played a role in enhancing chicken breast meat quality, which can be attributed to its role in reducing lipid peroxidation and improving antioxidant capacity.

The contents of lipid-metabolism-related products in the serum and liver of animals reflect the lipid metabolism function, which is closely intertwined with the growth, development, and metabolism of the animal. Extracts containing SAL components effectively decreased TG, TC, and LDL-C levels in rats while elevating HDL-C content (Luan et al., 2023). In our study, SAL supplementation increased HDL-C levels in broiler serum and liver and suppressed TG, TC, and LDL-C levels, which is beneficial for preventing metabolic disorders. Furthermore, we found that high-dose SAL had more pronounced effects on lipid metabolism

regulation compared to low-dose SAL, which is consistent with previous studies (Hu et al., 2021). The chemical composition of meat is a crucial determinant of its nutritive value (Gao et al., 2022). After SAL supplementation, crude fat content notably decreased in chicken breast meat, which suggested that SAL effectively reduced lipid accumulation and regulated overall body lipid metabolism.

The functionality of antioxidant enzymes is closely interconnected with the physiological response of the body to oxidative stress and its overall well-being. The antioxidant enzymes are significant parts of the body's antioxidant defense system; they include SOD, CAT, and GSH-Px. In our study, the increase in antioxidant enzyme activity may be caused by SAL supplementation. Nrf2, a crucial transcription factor involved in the cellular response to oxidative stress, interacts with the antioxidant response element (ARE) to modulate the expression of subsequent antioxidant genes (Huang, Nguyen, & Pickett, 2000). Our study revealed that SAL upregulated HO-1, GSH, CAT, and Nrf2, indicating the antioxidant effect of SAL to improve meat quality. A previous study reported that incorporating antioxidants into the chicken diet promotes the activity of antioxidant enzymes and augments the expression of genes associated with Nrf2, consequently refining the meat color, tenderness, and stability of the myofibrillar protein structure in chicken breast meat (Wang et al., 2023).

During the post-slaughter storage period, chicken breast meat is vulnerable to damage due to reactive oxygen species. This can result in lipid and protein oxidation, consequently leading to a decline in meat quality (Falowo, Fayemi, & Muchenje, 2014). MDA and TBARS are the products of lipid oxidation and can negatively affect meat quality and flavor. The carbonyl content in proteins serves as a crucial indicator of determining the extent of protein oxidation. Higher levels of DPPH, a stable nitrogen-centered free radical, indicate better scavenging ability and a stronger antioxidant capacity (Kumar et al., 2020). Incorporating plant extracts as feed additives enhanced the meat's antioxidant status in chicken, thus improving meat quality (Wang et al., 2023). Consistent with this, the findings of our study indicated that SAL increased the clearance rate of DPPH, reduced meat oxidation products (MDA, TBARS, and carbonyl), and significantly contributed to the enhancement of meat quality. Surprisingly, SAL supplementation did not affect the regulation of NEFA and T-AOC levels in chicken breast meat.

The fatty acid content and saturation present in food are intricately related to human health. Consumption of meats rich in PUFA, particularly n-3 UFA, has significant health benefits (Krauss et al., 2001). As far as we know, this is the first research to confirm that SAL changes the fatty acid makeup of chicken breast muscle. SAL supplementation resulted in the reduction in SFA and increase in UFA. The variations in fatty acid composition can be attributed to changes in lipid metabolism resulting from distinct dietary treatments (Kumar et al., 2020). However, the exact mechanism underlying the regulation of fatty acid content in chicken breast meat after SAL supplementation warrants further investigation. The imbalanced n-6: n-3 fatty acid ratio in diet significantly threatens human health, primarily by stimulating the excessive production of proinflammatory arachidonic acid, which triggers the release of inflammatory cytokines (Hotamisligil, 2006; Kumar et al., 2019). The n-6 PUFA content increases the risk of blood clot formation (Christophersen & Haug, 2011). In the finding of our study, the n-6: n-3 PUFA ratio decreased in chicken breast meat after SAL supplementation. This finding is in line with earlier research, which suggested that supplementing the diet with antioxidant components reduced SFA levels and n-6: n-3 PUFA ratio (Gao et al., 2022).

Increasing evidence shows that the composition and amounts of fatty acids present in meat significantly influence human cardiovascular health. Meat health indices offer a method for evaluating the impact of the fatty acid profile of diet on the development of chronic diseases. The introduction of AI and TI in 1991 presented a means to evaluate blood lipid health indices, revealing how fatty acid composition affects the cardiovascular system and coronary artery health (Ulbricht et al., 1991). Feeding broilers with a diet containing flaxseed and turmeric elevated the levels of PUFA and AI and TI values in chicken breast meat (Kumar et al., 2020). Consistent with this, our results demonstrated that SAL supplementation reduced the AI, TI, and HFA values. In addition, DFA, HPI, and NVI are essential markers for assessing the dietary meat's nutritional content (Chen et al., 2004). Our findings showed that SAL supplementation leads to increased values of DFA, NVI, and HPI in chicken breast meat, thus increasing its nutritive value.

The freezing of meat is crucial for storage and transportation. However, repeated freezing and thawing is unavoidable. Multiple freeze-thaw cycles affect meat quality and nutrition, which has garnered attention (Ali et al., 2015). Evidence shows that the color stability and water-holding capacity of chicken meat are significantly decreased and protein and lipid oxidation is increased after 5 freeze-thaw cycles (Ali et al., 2015). The influence of supplementing plant extracts in feed on the quality of repeatedly frozen chicken breast meat is rarely reported. Thus, we assessed whether SAL could enhance the meat quality after 5 freeze-thaw cycles. The results revealed that the addition of SAL to the chicken diet not only enhanced the quality of fresh chicken meat but also proved to be beneficial during storage and transportation.

The ratio of the weight of internal organs to that of the body reflects the development of various organs and the overall physiological functioning of animals to a certain extent. In our study, SAL supplementation increased the ratio of weights of chicken breast meat and leg meat to body weight, indicating a promotional effect of SAL on muscle growth. Unexpectedly, SAL supplementation did not significantly increase chicken body weight, possibly due to the reduction in the liver weight to body weight ratio. A study revealed that compared with laying hens on a high-fat diet, SAL supplementation resulted in reduced body weight, liver weight, and abdominal fat weight in laying hens (Cui et al., 2022). Furthermore, SAL exhibited no noticeable effect on the ratio of the weights of the spleen, heart, and bursa of Fabricius to body weight in chicken, suggesting no significant promotion or inhibition of the development of these organs.

Multiple studies suggested a correlation among lipid metabolism, antioxidant capacity, and meat quality in meat products (Huang et al., 2021; Y. J. Wang et al., 2023). Many components of meat are susceptible to oxidative reactions, primarily including proteins and lipids. Excessive oxidation of meat significantly affects its sensory attributes and nutritional profile (Bellucci, Bis-Souza, Domínguez, Bermúdez, & Barretto, 2022). Some natural extracts from plant sources act as antioxidants and contributes to maintaining meat quality (Bellucci et al., 2022). Antioxidants such as resveratrol (Meng et al., 2020) and curcumin (Zhang et al., 2015) are reported to significantly enhance meat quality, including color, pH, and water-holding capacity. In our study, Pearson's correlation analysis indicated that a strong association existed between antioxidant indicators and meat quality parameters. Because of the naturally low levels of antioxidants in chicken meat, lipid peroxidation occurs easily. Supplementing antioxidants in the feed could enhance the antioxidative capacity of broiler chicken, thereby improving meat color, water retention, tenderness, and other characteristics (Valentini et al., 2020; Wang et al., 2023). This effect was observed after SAL supplementation in our study. Our results revealed a clear correlation among meat quality parameters, crude fat content, and fatty acid profile. Targeted regulation of lipid metabolism and deposition can enhance meat quality and may have beneficial effects on human health (Dodson et al., 2010). A study demonstrated that high lipid levels in the breast muscle of duck meat lead to increased brightness, yellowness, and cooking loss (Chartrin et al., 2006). Meat containing higher lipid levels is more susceptible to lipid peroxidation when exposed to air, promoting the formation of toxic byproducts that may compromise health (Ribeiro et al., 2019). The process of lipid oxidation is complex, involving the participation of free radicals and reactive oxygen species (Bellucci et al., 2022). It is anticipated that reducing the lipid content of meat while simultaneously bolstering antioxidant capacity will ameliorate meat quality by mitigating lipid peroxidation. In our study, a potential correlation was observed between fatty acid profile and antioxidant parameters in breast

meat. The fatty acid profile of muscles is linked to antioxidative capacity and often collectively influences meat stability (Valentini et al., 2020). In summary, supplementing the chicken diet with SAL may enhance meat quality by promoting the antioxidative capacity of broiler chicken and modulating the fatty acid profile.

5. Conclusion

Our study demonstrated that supplementing SAL to the daily diet of broilers enhances the meat quality, reflected by meat color, pH, water retention, and shear force. Moreover, SAL supplementation increased antioxidant enzyme activity and UFA levels and decreased lipid peroxidation, crude fat content, and SFA content. The mechanism underlying the enhancement of chicken meat quality by SAL may involve the promotion of the antioxidant capacity and regulation of the fatty acid composition. Our findings provide the data supporting the inclusion of SAL in broiler chicken diets to enhance meat quality. More experiments are needed to investigate the application of SAL in improving meat quality in broiler farms, as well as its combined effects with other functional feed additives.

CRediT authorship contribution statement

Yanyan Zhang: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. Hongfan Ge: Methodology, Investigation. Yaling Yu: Methodology, Investigation. Hang Gao: Methodology. Xiaoli Fan: Methodology. Qiao Li: Writing – review & editing. Zhenlei Zhou: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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