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Effects of dietary supplementation with microencapsulated *Galla chinensis* tannins on growth performance, antioxidant capacity, and lipid metabolism of young broiler chickens

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This study aimed to investigate the impacts of dietary supplementation with *Galla chinensis* tannins (GCT) on the growth performance, antioxidant capacity, and lipid metabolism of young broilers. Overall, a total of 216 healthy 1day-old broilers were randomly allocated to CON group and GCT group, and provided with a basal diet or a basal diet added with 300mg/kg microencapsulated GCT, respectively, in a 21days trial. Our findings indicated that dietary GCT addition had no significant effects ($p>0.05$) on growth performance. However, GCT supplementation led to a significant reduction in the total cholesterol (TC) concentration in the serum and liver ($p<0.05$). Furthermore, GCT supplementation significantly increased the ratios of high-density lipoprotein (HDL) to low-density lipoprotein (LDL) and HDL to TC in the serum, in addition to elevating the activities of enzymes related to lipid metabolism in the liver ($p<0.05$). Dietary GCT addition also improved the antioxidant capacity of the broilers, as evidenced by a significant decrease in the concentration of malondialdehyde in serum and liver ($p<0.05$). Additionally, the GCT group exhibited significantly increased expressions of hepatic genes associated with antioxidant enzymes (*HO-1*, *GPX1*, *SOD2*, *SIRT1*, *CPT-1*, and *PPAR α*) ($p<0.05$), while the mRNA expression of *SREBP-1* was significantly decreased ($p<0.05$) compared with the CON group. In conclusion, dietary addition of 300mg/kg microencapsulated GCT improved the antioxidant status and lipid metabolism of broilers without affecting their growth performance.

KEYWORDS

antioxidant capacity, broiler, *Galla Chinensis*, lipid metabolism, tannic acid

Introduction

With the ongoing intensification of the poultry industry in the modern world, broilers are confronted with a multitude of challenges including infections, oxidative stress, and lipid accumulation (1, 2). These factors can disrupt the balance between oxidation and the antioxidant defense system, thereby affecting the growth performance of broilers (3, 4). Furthermore, the body's antioxidant efficiency is closely associated with lipid metabolism. The liver, as the main metabolic organ, plays a crucial role in both the antioxidant defense mechanism and lipid metabolism. Fatty acids obtained from the gastrointestinal tract are transported directly to the liver, where over 90% of the broilers' body fat is synthesized (5, 6). Excessive hepatic lipid accumulation can lead to severe metabolic disorders, tissue damage, and even mortality (7). Young broiler chickens (1–21 days of age) particularly susceptible to oxidative stress due to the incomplete development of their antioxidant system (8). Therefore, it is crucial to regulate antioxidant efficiency and lipid metabolism to maintain good health and prevent diseases in young broiler chickens (9, 10).

Tannins are plant secondary metabolites that can be classified into hydrolyzable tannins and condensed tannins, and they are present in various plant components including seeds, flowers, leaves, roots, and fruits (11, 12). *Galla chinensis* (GC), renowned in traditional Chinese medicine for centuries, contains hydrolyzable tannins as its primary bioactive compound (13). According to reports, tannins possess numerous biological properties, including antibacterial, anti-parasitic, antioxidative, anti-inflammatory, and antiviral actions (14). Tannins are highly effective and safe, causing minimal side effects and toxicity. As a result, they are frequently employed in the prevention and treatment of lipid metabolism disorders (15). Nevertheless, tannins may reduce feed intake by reducing the palatability (16). Microencapsulation is a widely used technique in the feed industry that effectively alters the absorption site of additives and conceals undesirable tastes and odors. A previous study in weaning piglets demonstrated that supplementing microencapsulated GC tannins (GCT) at doses of 500–1,000 mg/kg had beneficial effects on intestinal development and function as well as the proliferation of beneficial bacteria proliferation (17). Furthermore, supplementation of microencapsulated GCT at a dose of 300 mg/kg demonstrated several beneficial effects in broilers, representing as improving growth performance, enhancing liver function, and providing protection against lipopolysaccharide-induced liver damage by inhibiting the TLR4/NF- κ B pathway (18, 19). However, there is limited scientific literature available on the effects of dietary GCT addition on the growth performance, antioxidant capacity, and lipid metabolism of young broiler chickens.

Therefore, this study aimed to investigate the impacts of dietary addition with GCT on growth performance, antioxidant capacity, and lipid metabolism of young broilers, supplying important knowledge on the role and mechanisms of microencapsulated GCT in regulating antioxidant capacity and lipid metabolism in poultry production.

Materials and methods

Animals and treatments

A total of 216 healthy Arbor Acres (AA) chickens (1 day of age) with an initial body weight (BW) of 48.94 ± 0.34 g were randomly

assigned to two dietary groups (6 replicates per group and 18 birds each replicate) in a 21 d trial. The experimental diets included a basal diet (CON group) and a basal diet added with GCT at a dose of 300 mg/kg (GCT group), according to the procedure described in previous studies (18, 19). The GCT used in this study, with an effective tannin concentration of 40%, was supplied by the Wufeng Chicheng Biotechnology Co., Ltd. (Yichang, China). In order to improve diet palatability and reduce potential irritation caused by tannins, microencapsulation was employed to mitigate undesirable effects (17). The basal diet (Table 1) was formulated in accordance with the nutrient requirements established by the National Research Council (NRC, 1994) (20). All the broilers were kept in a mental chicken coop (three-tiered cages) positioned in an environment-controlled room

TABLE 1 Ingredients composition and nutrient levels of basal diets (as-fed basis).

Items	Content
Ingredients, %	
Corn	55.91
Soybean meal, 44% CP	13.78
Wheat bran	11.98
Corn starch residue	7.99
Corn gluten meal	3.99
Extruded soybean	1.50
Limestone	1.70
Calcium monophosphate	1.10
L-Lysine HCl, 76.8%	1.00
DL-Methionine, 98%	0.20
L-Threonine, 98%	0.10
Sodium chloride	0.40
Choline	0.10
Phytase	0.10
Complex enzyme	0.02
Trace mineral premix ^a	0.10
Vitamin premix ^b	0.02
Antioxidant	0.02
Total	100
Calculated analysis, %	
Metabolizable energy, MJ/kg	12.33
Crude protein	19.47
Crude fat	3.45
Calcium	0.94
Available phosphorus	0.35
Lysine	1.15
Methionine	0.50

^aProvided per kilogram of complete basal diet: 10 mg of Cu as CuSO₄, 100 mg of Fe as FeSO₄, 1.1 mg of I as Ca(IO₃)₂, 65 mg of Zn as ZnSO₄, 100 mg of Mn as MnSO₄ and 0.3 mg of Se as Na₂SeO₃.

^bProvided per kilogram of complete basal diet: vitamin A 10,000 IU, vitamin D₃ 3,000 IU, vitamin E 30 IU, menadione 1.3 mg, thiamine 2.2 mg, riboflavin 8 mg, pyridoxine 4 mg, vitamin B₁₂ 0.025 mg, D-biotin 0.2 mg, niacin 40 mg, folic acid 1 mg, and D-calcium pantothenate 10 mg.

and under constant lighting. The temperature in the room was kept at 32°C for the first 3 days before dropping by 1°C every other day thereafter. The weight of the broiler chickens was measured at the beginning and end of the experiment, and daily feed intake were recorded during the experiment. Average daily gain, average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated as described in previous study (18).

Samples collection

On day 21, one broiler in each replicate (cage) with a BW close to the average of the replicate were selected after a 12 h fasting period. Blood samples were collected from the wing vein into the vacuum tubes, and the serum was then obtained by centrifugation (3,500 × g, 15 min), followed by being stored at −20°C for further analysis. Subsequently, the broilers were euthanized by cervical dislocation and eviscerated. Approximately 5 g of liver samples were collected and stored at −80°C after quick-freezing in liquid nitrogen; and another part was fixed with 4% paraformaldehyde solution for 24 h at room temperature after being rinsed with normal saline.

Determination of serum biochemical parameters

The serum concentrations of total protein (TC), triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were examined using an automatic biochemical analyzer (COBUS MIRA Plus, Roche Diagnostic System Inc., United States) with commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China).

Determination of antioxidant capacity

Samples of liver tissues were homogenized with ice-cold 0.9% sodium chloride (1,10, w/v), and centrifuged at 4,000 rpm at 4°C for 10 min to obtain clarified homogenates. Activities of glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD), and catalase (CAT), as well as the levels of total antioxidative capacity (T-AOC) and malondialdehyde (MDA), in serum and liver samples were determined using kits purchased from Jiancheng Bioengineering Institute in accordance with the methods described by Chen et al. (21). The concentration of liver hydrogen peroxide (H₂O₂) was assayed using commercial kits (Beyotime Biotechnology, Shanghai, China) following the manufacturer's protocol (22).

Liver morphological analysis

After 24 h fixation with 4% paraformaldehyde solution, the liver tissues were dehydrated with graded concentrations of ethyl alcohol, and embedded in liquid paraffin (23). Then 5 μm slices of the liver tissue were cut and stained with hematoxylin and eosin (H&E) after being embedded in paraffin wax. And liver sections were observed under an Olympus digital microscope (Olympus BX51, Tokyo, Japan).

Determination of liver lipid metabolism-related parameters

The levels of triglyceride (TG), total cholesterol (TC), total lipase (TL), lipoprotein lipase (LPL), and hepatic lipase (HL) in liver homogenate samples were assayed using the commercial kits purchased from the Jiancheng Bioengineering Institute. The activities of fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), and hormone-sensitive TG lipase (HSL) in hepatic were determined using kits from Yili Biological Technology Co., Ltd. (Shanghai, China).

Gene expression

The TRIzol Reagent (Invitrogen, Carlsbad, CA, United States) was used to extract the total RNAs from the frozen liver samples according to the manufacturer's instructions. A reverse transcription kit (TaKaRa, Dalian, China) was used to synthesize cDNA, which was then amplified by quantitative real-time PCR using SYBR Premix Ex Taq Reagents (TaKaRa). The primer sequences were displayed in Table 2, and β-actin was quantified in parallel as the internal control for normalization and quantification of transcription levels. The PCR cycling conditions were set as described in a previous study (3). The 2^{−ΔΔCt} method was applied to determine the relative abundances of the mRNA of the detected genes in liver samples.

Statistical analysis

The replicate was regarded the experimental unit to evaluate effects on growth performance, while individual broiler was regarded the experimental unit for other data analyses. Statistical analysis of the data was performed using a *t*-test in SAS 9.4 (Institute Inc., Cary, NC, United States). Normality of the data was evaluated by the Shapiro–Wilk statistic ($W > 0.05$). Results are expressed as the mean ± standard error and as plots or graphs. Statistically differences between the two groups were regarded at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, while # $p < 0.10$ was considered indicative of a trend toward significance.

Results

Growth performance

Effects of dietary microencapsulated GCT addition on the growth performance of broilers are displayed in Figure 1. Throughout the 21 days trial, no significant differences in ADFI (Figure 1A), ADG (Figure 1B), or FCR (Figure 1C) between broilers in the CON and GCT groups ($p > 0.05$).

Serum biochemical parameters

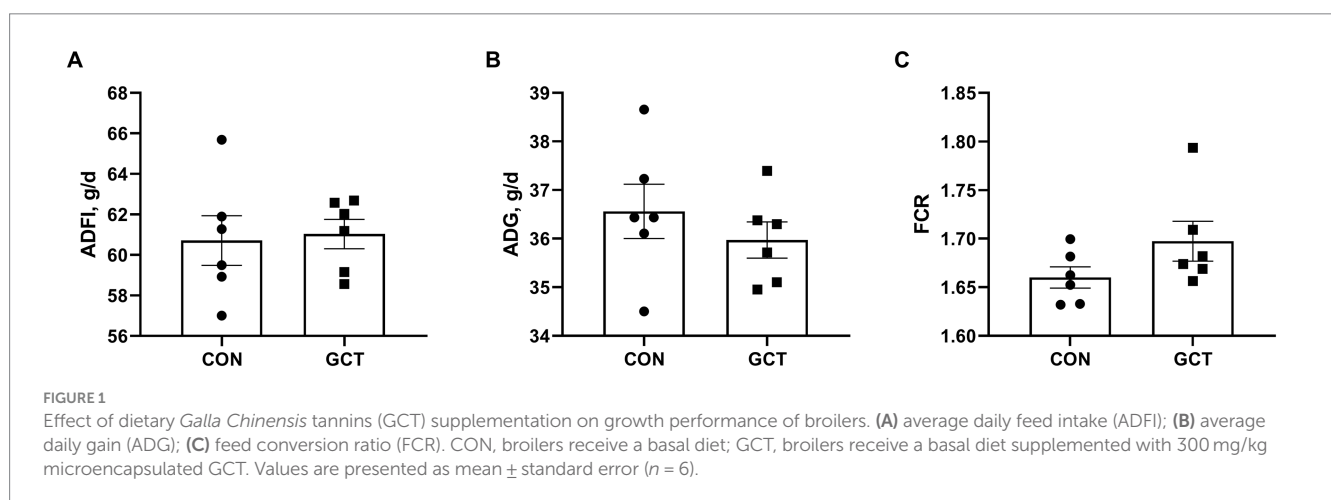
As shown in Figure 2, the serum concentrations of TC (Figure 2A) and LDL (Figure 2D) in the GCT group were significantly lower than those in the CON group ($p < 0.05$). Additionally, the GCT group exhibited significantly higher HDL/LDL ratio (Figure 2E) and HDL/TC ratio (Figure 2F) compared with

TABLE 2 Primer sequences used for quantitative real-time PCR.

Genes ^b	GenBank	Primer sequences ^a , 5'-3' ¹	Size, bp
<i>β-actin</i>	NM_205518.1	F:ATTGTCCACCGCAAATGCTTC R:AAATAAAGCCATGCCAATCTCGTC	113
<i>NRF2</i>	XM_015289381.2	F:CCCGCACCATGGAGATCGAG	180
		R:GGAGCTGCTCTTGTCTTTCCT	
<i>HO-1</i>	NM_205344.1	F:GTCGTTGGCAAGAAGCATCC	106
		R:GGCCTTTTGGGCGATTTC	
<i>SOD1</i>	NM_205064.2	F:CGCAGGTGCTCACTTCAATCC R:CAGTCACATTGCCGAGGTCAC	89
<i>SOD2</i>	NM_204211.2	F:GCTGTATCAGTTGGTGTCAAGGA R:GCAATGGAATGAGACCTGTTGTTC	130
<i>CAT</i>	NM_001031215.2	F:GGAGGTAGAACAGATGGCGTATG R:CGATGCTATGCGTGTGAGGAT	114
<i>GPX1</i>	NM_001277853.3	F:CGGCTTCAAACCCAACCTTCAC R:CTCTCTCAGGAAGGCGAACAG	85
<i>SIRT1</i>	XM_046920057.1	F:GATCAGCAAAAGGCTGGATGGT	143
		R:ACGAGCCGCTTTCGCTACTAC	
<i>CPT-1</i>	NM_001012898.1	F:ACAGCGAATGAAAGCAGGGT	93
		R:GCCATGGCTAAGGTTTTCGT	
<i>PPARα</i>	NM_001001464.1	F:AGTAAGCTCTCAGAACTTTGTTG	161
		R:AGGTTGAAACAGAAGCCGC	
<i>APOA1</i>	XM_046932309.1	F:CGCATTCCGGATATGGTGGGA	102
		R:GTCAAGCTGTTTGCCACAG	
<i>SREBP-1</i>	AY029224	F:GCAGAAGAGCAAGTCCCTCAA	104
		R:TCGGCATCTCCATCACCTC	

^aF, forward primer; R, reverse primer.

^bSIRT1, sirtuin1; NRF2, nuclear factor erythroid 2-related factor 2; HO-1, heme-oxygenase 1; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; CAT, catalase; GPX1, glutathione peroxidase 1; CPT-1, carnitine palmitoyl-transferase 1; PPARα, peroxisome proliferator-activated receptor α; APOA1, apolipoprotein A1; SREBP-1, sterol regulatory element-binding protein-1.



the CON group ($p < 0.05$). No significant differences were observed in serum TG (Figure 2C) and HDL (Figure 2D) concentrations between the two groups ($p > 0.05$).

Serum and liver antioxidant capacity

Effects of GCT addition on serum antioxidant parameters of broilers are presented in Figure 3. Broilers in the GCT group showed a significantly higher ($p < 0.05$) T-AOC (Figure 3D) level and a lower

($p < 0.05$) MDA (Figure 3E) level. Moreover, there was a trend towards higher serum T-SOD (Figure 3B) and GSH-Px (Figure 3C) activities in the GCT group compared to the CON group ($p < 0.10$). There was no significant difference in serum CAT (Figure 3A) activity ($p > 0.05$).

Effects of GCT addition on hepatic antioxidant parameters of broilers are shown in Figure 4. Dietary 300 mg/kg microencapsulated GCT addition significantly elevated ($p < 0.05$) T-SOD (Figure 4B) and T-AOC (Figure 4D) levels, while significantly decreased ($p < 0.05$) MDA (Figure 4E) and H_2O_2 (Figure 4F) concentrations in

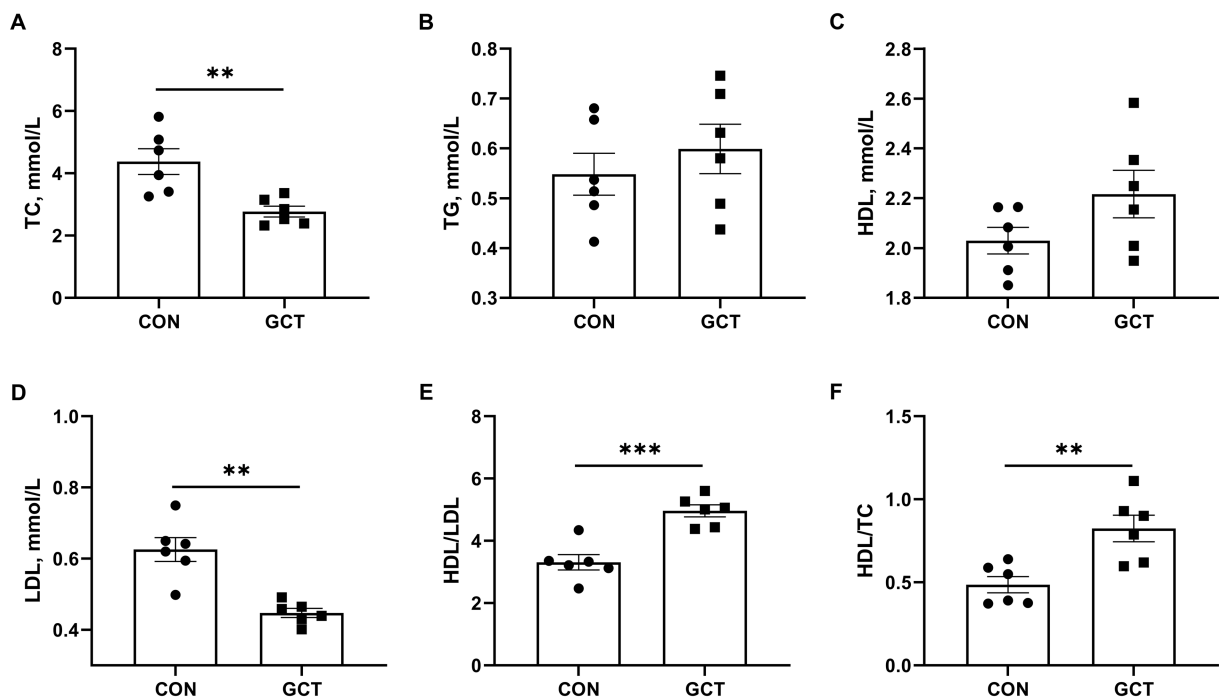


FIGURE 2

Effects of dietary *Galla Chinensis* tannins (GCT) supplementation on serum biochemical parameters of broilers. (A) total cholesterol (TC); (B) triglyceride (TG); (C) high-density lipoprotein (HDL); (D) low-density lipoprotein (LDL); (E) HDL/LDL ratio; (F) HDL/TC ratio. CON, broilers receive a basal diet; GCT, broilers receive a basal diet supplemented with 300 mg/kg microencapsulated GCT. Values are presented as mean \pm standard error ($n = 6$). ** $p < 0.01$ and *** $p < 0.001$.

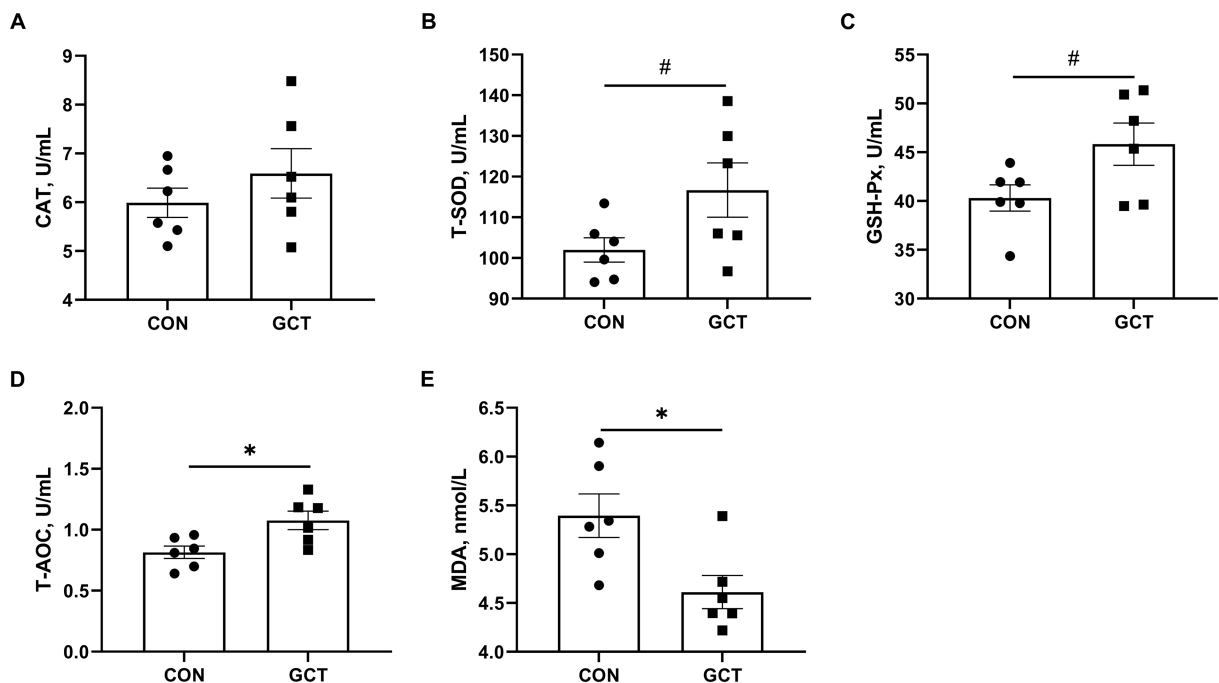
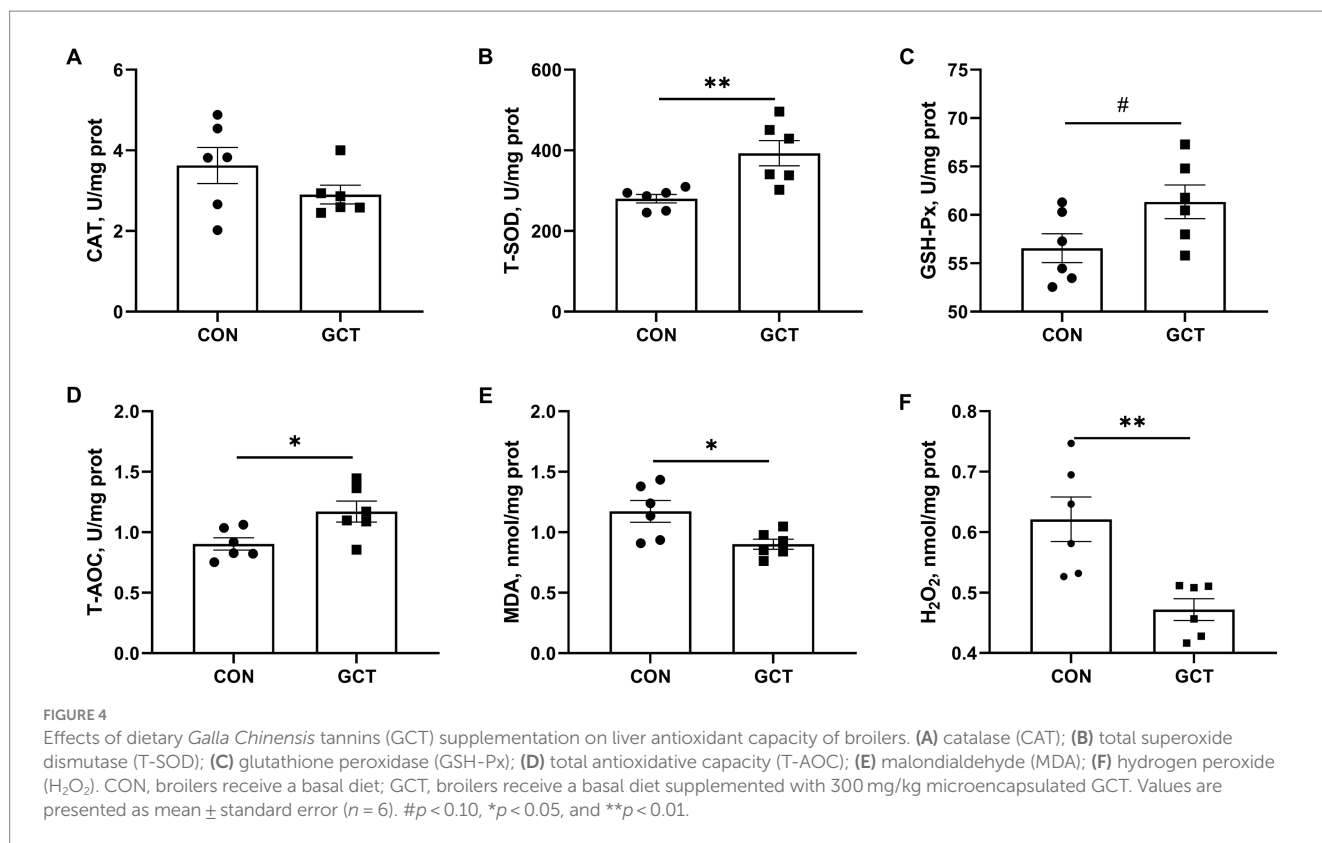


FIGURE 3

Effects of dietary *Galla Chinensis* tannins (GCT) supplementation on serum antioxidant capacity of broilers. (A) catalase (CAT); (B) total superoxide dismutase (T-SOD); (C) glutathione peroxidase (GSH-Px); (D) total antioxidative capacity (T-AOC); (E) malondialdehyde (MDA). CON, broilers receive a basal diet; GCT, broilers receive a basal diet supplemented with 300 mg/kg microencapsulated GCT. Values are presented as mean \pm standard error ($n = 6$). # $p < 0.10$, * $p < 0.05$, and ** $p < 0.01$.



the liver of broilers. Moreover, microencapsulated GCT supplementation showed a tendency toward increased ($p < 0.10$) hepatic GSH-Px activity (Figure 4C). There was no significant difference observed in liver CAT (Figure 4A) activity between the two groups ($p > 0.05$).

Liver lipid metabolism

In the liver, the GCT group exhibited significantly lower ($p < 0.05$) TC (Figure 5A) level and significantly higher ($p < 0.05$) activities of HL (Figure 5D), TL (Figure 5E), and HSL (Figure 5H) relative to the CON group. Furthermore, GCT supplementation to broiler diet showed a trend ($p < 0.10$) toward reduced liver TG (Figure 5B) level compared with CON broilers. Dietary GCT supplementation had no remarkable influences ($p > 0.05$) on the activities of LPL (Figure 5C), ACC (Figure 5F), and FAS (Figure 5G) in the liver.

Liver histomorph

As shown in Figure 6, the liver structure of the CON group and GCT group appeared intact, characterized by well-organized liver cell cords and normal morphology of liver cells. The bile duct structure in the portal area was clear, without any observed changes such as bile duct proliferation, lumen enlargement, or inflammatory cell infiltration. However, in the GCT group, the liver sinus appeared thinner and more compact, accompanied by a noticeable reduction in liver tissue fat globules and a tendency towards decreased sphericity.

Expressions of hepatic antioxidant-related genes

Effects of GCT addition on relative expressions of hepatic antioxidant-related genes of broilers are shown in Figure 7. The GCT group exhibited significantly upregulated ($p < 0.05$) mRNA expressions of *HO-1* (Figure 7B), *GPX1* (Figure 7D), and *SOD2* (Figure 7F) compared in the CON group (Figure 6). However, dietary GCT supplementation had no significantly affects ($p > 0.05$) on the mRNA expressions of *NRF2* (Figure 7A), *CAT* (Figure 7C), and *SOD1* (Figure 7E).

Expressions of hepatic lipid metabolism-related genes

As shown in Figure 8, dietary microencapsulated GCT supplementation significantly upregulated ($p < 0.05$) the hepatic mRNA expressions of *SIRT1* (Figure 8A), *CPT-1* (Figure 8B), and *PPAR α* (Figure 8C), while significantly downregulated ($p < 0.05$) the mRNA expression of *SREBP-1* (Figure 8E) in broilers. No significant difference was found ($p > 0.05$) in hepatic *APOA1* (Figure 8D) mRNA expression between CON and GCT groups.

Discussion

In current study, dietary supplementation with 300 mg/kg microencapsulated GCT did not show negative effect on the growth performance of young broilers, which was in accordance with previous

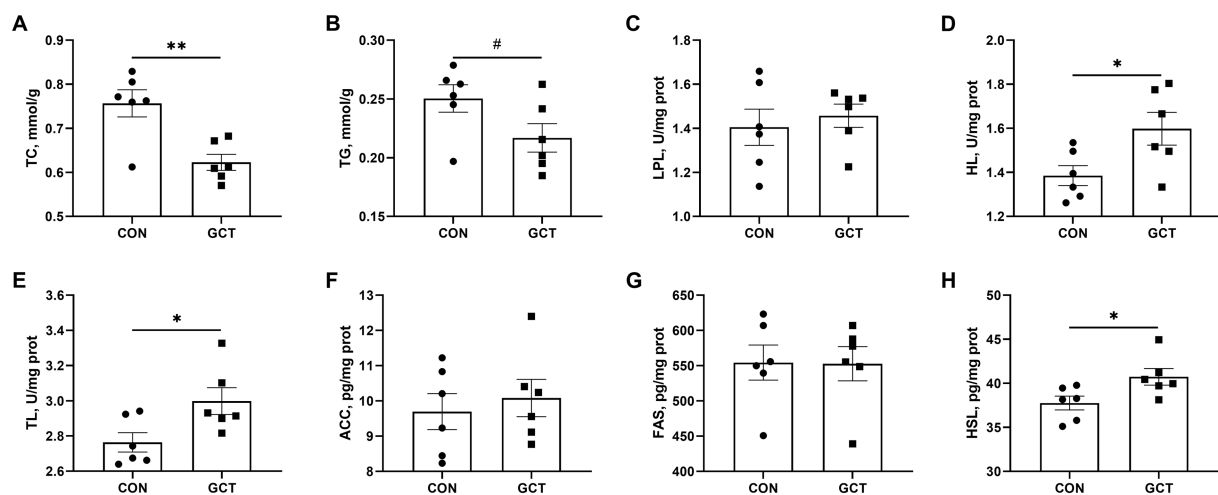


FIGURE 5

Effects of dietary *Galla Chinensis* tannins (GCT) supplementation on liver lipid metabolism of broilers. (A) total cholesterol (TC); (B) triglyceride (TG); (C) lipoprotein lipase (LPL); (D) hepatic lipase (HL); (E) total lipase (TL); (F) acetyl CoA carboxylase (ACC); (G) fatty acid synthase (FAS); (H) hormone-sensitive triglyceride lipase (HSL). CON, broilers receive a basal diet; GCT, broilers receive a basal diet supplemented with 300 mg/kg microencapsulated GCT. Values are presented as mean \pm standard error ($n = 6$). # $p < 0.10$, * $p < 0.05$, and ** $p < 0.01$.

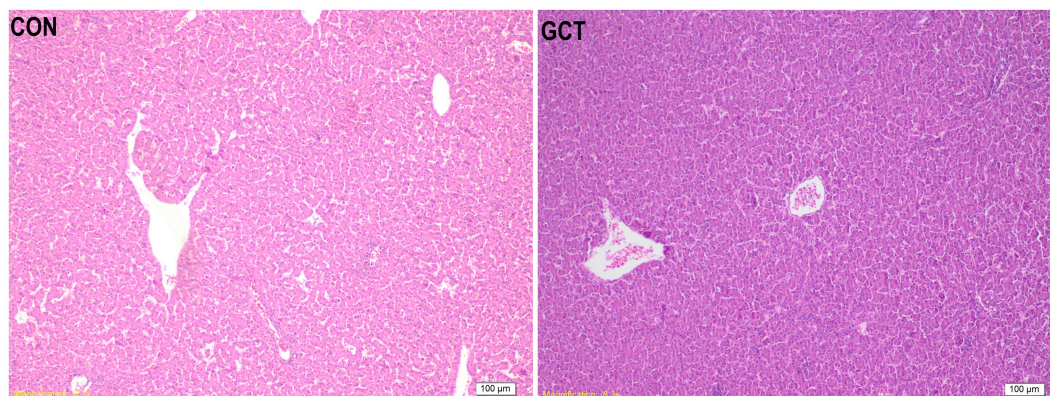


FIGURE 6

Hematoxylin and eosin photomicrographs of liver tissues. CON, broilers receive a basal diet; GCT, broilers receive a basal diet supplemented with 300 mg/kg microencapsulated GCT.

studies (18, 24). Tannins in higher concentration are antinutritional because made chelates and reduce protein digestibility (25). It was reported that tannins could form complexes with proteins (both enzymes and nonenzyme proteins) to form tannin-protein complexes, reducing the bioavailability of nutrients in the feed (26). Buyse et al. (24) reported that broilers fed a diet containing higher dose (2000 mg/kg) of chestnut tannins had lower performance during the grower and finisher phases than broilers fed a diet with a lower dose (500 mg/kg) of chestnut tannins. These results indicated that low concentrations of tannins had no adverse effects on broiler growth performance. Additionally, tannins have poor palatability for livestock. Microencapsulation is widely used in the feed industry to modify the absorption site and mask undesirable taste and odor (17). In piglets, dietary supplementation of microencapsulated tannins extracted from GC had no adverse effects on growth performance (27). Furthermore, Niu et al. (18) indicated that 300 mg/kg microencapsulated CGT

improved the feed conversion ratio of broilers throughout the trial, which could be attributed to the improved intestinal development after 42 days of feeding (28). Therefore, supplementing 300 mg/kg microencapsulated CGT in the diet had no adverse effects on the growth performance of young broiler chickens.

The liver, a vital metabolic organ, plays an important role in metabolism and defense against bacterial invasion and bacterial products (22). It contains a large number of mitochondria and acts as a regulator of energy balance with high oxygen consumption and reactive oxygen species production (29). Intensive farming practices in poultry production have increased the likelihood of hepatic oxidative injury because of higher metabolic demands during the growth and development of broilers (30). Therefore, the implementation of measures is necessary to improve the antioxidant capacity of the liver. In the current study, supplementation of 300 mg/kg GCT elevated T-SOD and GSH-Px activities, as well as T-AOC

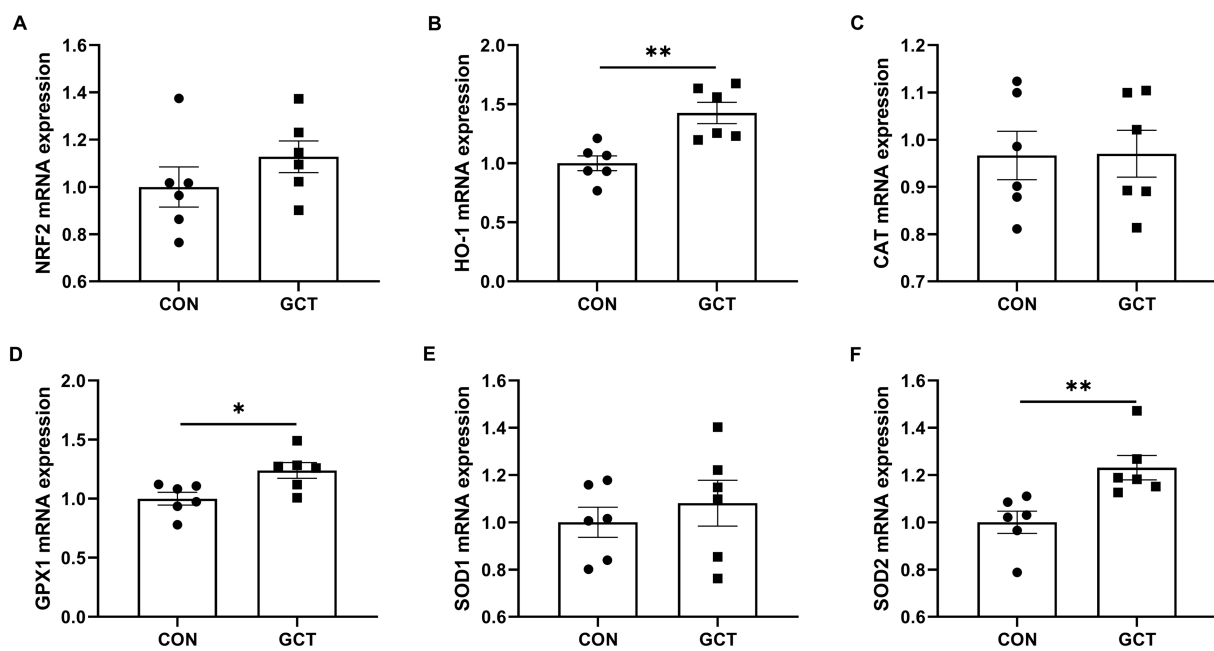


FIGURE 7

Effects of dietary *Galla Chinensis* tannins (GCT) supplementation on mRNA expressions of hepatic antioxidant-related genes of broilers. (A) nuclear factor erythroid 2-related factor 2 (NRF2); (B) heme-oxygenase 1 (HO-1); (C) catalase (CAT); (D) glutathione peroxidase 1 (GPX1); (E) superoxide dismutase 1 (SOD1); (F) superoxide dismutase 2 (SOD2). CON, broilers receive a basal diet; GCT, broilers receive a basal diet supplemented with 300 mg/kg microencapsulated GCT. Values are presented as mean \pm standard error ($n = 6$). * $p < 0.05$ and ** $p < 0.01$.

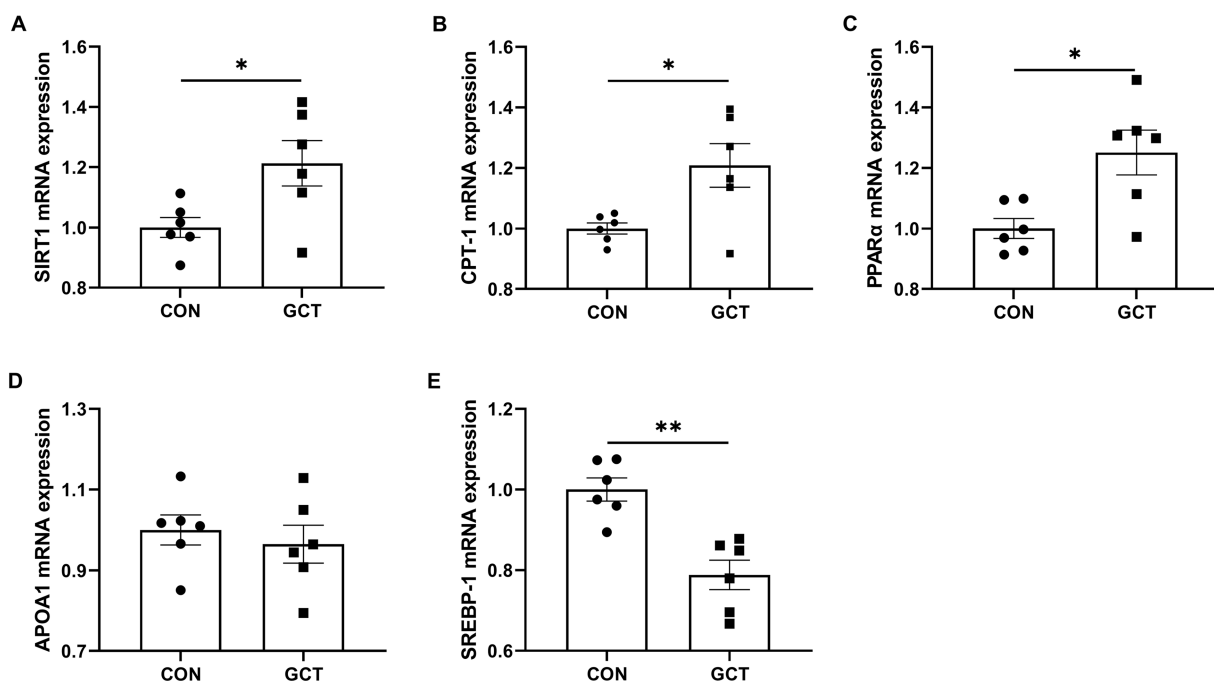


FIGURE 8

Effects of dietary *Galla Chinensis* tannins (GCT) supplementation on mRNA expressions of hepatic lipid metabolism-related genes of broilers. (A) Sirtuin1 (SIRT1); (B) carnitine palmitoyl-transferase 1 (CPT-1); (C) peroxisome proliferator-activated receptor α (PPAR α); (D) apolipoprotein A1 (APOA1); (E) sterol regulatory element-binding protein-1 (SREBP-1). CON, broilers receive a basal diet; GCT, broilers receive a basal diet supplemented with 300 mg/kg microencapsulated GCT. Values are presented as mean \pm standard error ($n = 6$). * $p < 0.05$ and ** $p < 0.01$.

levels, and decreased MDA levels in the serum and liver. Additionally, it led to a reduction in H_2O_2 concentration specially within the liver. Antioxidant enzymes, such as SOD, GSH-Px, and CAT, play vital roles

in preventing oxidative damage (22). The SOD converts ROS to the H_2O_2 , which can then be degraded into water and oxygen by GSH-Px and CAT (31). T-AOC is an important integrative index reflecting the

total antioxidant status (32), while MDA is an important indicator of detecting the degree of lipid peroxidation of the body (33, 34). Higher concentrations of H₂O₂ and MDA are typically associated with cellular damage. A previous study indicated that tannic acid improved CAT, SOD, and GSH-Px activities in rats challenged by arsenic trioxide (35). Song et al. (36) found that dietary supplementation of GCT improved intestinal antioxidant capacity in weaned piglets. Furthermore, GCT supplementation upregulated the mRNA expression of *HO-1* in the liver of broilers in the current study. HO-1, which is induced as a crucial stress protein, regulates many antioxidant enzymes and proteasomes and plays a major role in regulating intracellular ROS levels to mitigate cellular oxidative stress response due to its antioxidant effects (37, 38). Consistently, hepatic GPX1 and SOD2 expressions were upregulated in CGT broilers. GPX1 and SOD2 are genes that encode the antioxidant enzymes GSH-Px and SOD, respectively. Above all, our findings demonstrated that dietary supplementation of 300 mg/kg microencapsulated GCT enhanced the antioxidant capacity of young broilers through upregulating the HO-1 expression and improving the activities of antioxidant enzymes.

Antioxidant capacity of the body is generally associated with lipid metabolism. Our study indicated that dietary GCT addition reduced the concentrations of TC and LDL, and increased the HDL/LDL ratio and HDL/TC ratio in the serum. Other researchers also found that tannins supplementation decreased serum levels of LDL, TC, and TG, but increased serum level of HDL (39, 40). It is well known that low blood HDL level and high TG, TC, and LDL levels are the major risk factors for cerebrovascular and cardiovascular diseases (41). Excessive accumulation of TG and TC is often associated with liver damage (42). The HDL and LDL are apolipoproteins that represent different forms of lipid transport in the blood. Specifically, HDL facilitates the transportation of excess cholesterol from the surrounding tissues back to the liver for eventual excretion from the body, while LDL transports cholesterol from the liver to various tissues of the body (43, 44). Therefore, the serum HDL/LDL ratio and HDL/TC ratios serve as reliable indicators of lipid accumulation. Not surprisingly, lower TC concentration in the liver was found in GCT broilers compared with the CON broilers, which might be also related to the increased TL, HL, and HSL activities in the liver. Studies have demonstrated that HL facilitates the absorption of unesterified cholesterol that has accumulated in HDL, and also plays a role in catalyzing the breakdown of TG into fatty acids (45). The HSL, serving as a key enzyme in fat breakdown, accelerates the hydrolysis of TG to glycerol and fatty acids (46). Kwon et al. (47) indicated that GC extract exhibited potent inhibitory activity against lipid accumulation in the pancreas *in vitro*. Zou et al. (48) also showed that high molecular weight persimmon tannin administration decreased serum TG and free fatty acids concentrations, increased the excretion of TG and TC, and improved hepatic steatosis in rats fed with a high-fat diet. Above all, our findings suggested that supplementation of 300 mg/kg microencapsulated GCT in the diet benefited to increase lipid synthesis and accelerate lipolysis in young broilers.

Lipid accumulation is a complex process regulated by numerous gene expression alterations that control lipolysis and lipogenesis (49). The SIRT1, CPT-1, and CPT-2 play a major role in fatty acid β -oxidation. Our study showed that dietary GCT addition significantly upregulated hepatic *SIRT1* and *CPT-1* mRNA expression in broiler chickens. SIRT1 governs the regulation of PGC-1 α , which subsequently modulates fatty acid oxidation and facilitates fatty acid

catabolism in the liver (50, 51). CPT-1 is considered a rate-limiting enzyme involved in transporting fatty acids into the mitochondria for oxidation (52, 53). Besides, GCT enhanced the *PPAR α* mRNA expression and weakened *SREBP-1* mRNA expression in our study. PPAR α is a ligand-activated nuclear receptor, which mainly influences fatty acid metabolism and modulates the lipid accumulation through increasing LPL expression (54, 55). SREBP-1 is a transcriptional regulator of genes involved in fatty acid and TG syntheses, and it catalyzes the transcription of the key genes including FAS and ACC (56, 57). Similarly, Zou et al. (48) also demonstrated that high molecular weight persimmon tannin decreased expression of FAS, SREBP-1, and ACC, increased the expression of CPT-1, and stimulated AMP-activated protein kinase phosphorylation in the liver of rats fed a high-fat diet. These results revealed that microencapsulated GCT might improve liver lipid accumulation by activating the SIRT1/SREBP-1 pathway and increasing the expression of genes related to fatty acid β -oxidation.

Conclusion

In conclusion, dietary supplementation with 300 mg/kg microencapsulated GCT enhanced the antioxidant capacity and improved the lipid metabolism of young broilers although had no effects on the growth performance. These findings provide valuable references for the utilization of GCT to alleviate oxidative stress and lipid metabolic disorders, and support the utilization of tannins extracted from GC in the poultry industry.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal studies were approved by the Animal Care and Use Committee of Shandong Agricultural University (protocol code SDAUA-2022-073). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

XR: Conceptualization, Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft. PY: Conceptualization, Data curation, Formal analysis, Software, Visualization, Writing – review & editing. JN: Data curation, Investigation, Methodology, Project administration, Writing – review & editing. YLiu: Data curation, Investigation, Methodology, Project administration, Writing – review & editing. YLi: Conceptualization, Methodology, Resources, Validation, Writing – review & editing. LH: Methodology, Resources, Writing – review & editing. SJ: Investigation,

Validation, Writing – review & editing. NJ: Formal analysis, Writing – review & editing. XY: Visualization, Writing – review & editing. JL: Funding acquisition, Investigation, Resources, Supervision, Writing – review & editing. WY: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

XR and JL were employed by Shandong Taishan Shengliyuan Group Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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