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Effects of intermittent cold stimulation on growth performance, meat quality, antioxidant capacity and liver lipid metabolism in broiler chickens

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ABSTRACT Intermittent cold stimulation (**ICS**) enhances broilers' resistance to cold stress. Nonetheless, further research is needed to investigate the underlying mechanisms that enhance cold stress resistance. A total of 160 one-day-old male Ross 308 broilers were randomly divided into 2 groups (CC and CS5), with the CC group managing temperature according to the standard for broiler growth stages, while the CS5 group were subjected to cold stimulation at a temperature 3℃ lower than the CC group for 5 h, every 2 d from 15 to 35 d. Sampling was conducted at 36 d (**36D**), 50 d (**50D**) and after acute cold stress for 24 h (**Y24**). First, we examined the effects of ICS on broiler growth performance, meat quality, antioxidant capacity, and lipid metabolism. The results demonstrated that ICS enhanced the performance of broilers to a certain degree. Specifically, the average weight gain in the CS5 group was significantly higher than that of the CC group, and the feed conversion ratio significantly decreased compared to CC at 4 W and 6 W ($P \leq 0.05$). Compared with the CC group, cold stimulation significantly reduced drip loss, shearing force, and yellowness (a* value) of chicken meat, while significantly increased redness (b* value) ($P \leq 0.05$). At Y24, the levels of T-AOC and GSH-PX in the serum of the CS5 group were significantly higher than those of the CC group, while the level of MDA was significantly lower ($P \leq 0.05$). The content of TG, FFA, and VLDL in the serum of the CS5 group was significantly elevated, whereas the level of TC and HDL was significantly lower ($P \leq 0.05$). In addition, we further explored whether AMPK-mTOR pathway is involved in the regulation of changes in lipid metabolism and the possible regulatory mechanisms downstream of the signaling pathway. The results showed that ICS significantly upregulated the expression levels of *AMPK* mRNA and protein in the liver of the CS5 group at 36D and Y24, while significantly down-regulating *mTOR* ($P \leq 0.05$). Compared with the CC group, ICS significantly down-regulated the mRNA expression levels of lipid synthesis and endoplasmic reticulum stress-related genes (*SREBP1c, FAS, SCD, ACC, GRP78* and *PERK*) at 36D and Y24, while significantly up-regulating the mRNA expression levels of lipid decomposition and autophagy-related genes (*PPAR* and *LC3*) ($P \leq 0.05$). In addition, at Y24, the protein expression levels of endoplasmic reticulum stress-related genes (*GRP78*) in the CS5 group were significantly lower, while autophagy-related genes (*LC3* and *ATG7*) were significantly higher ($P \leq 0.05$). ICS can affect meat quality and lipid metabolism in broilers, and when broilers are subjected to acute cold stress, broilers trained with cold stimulation have stronger lipid metabolism capacity.

Key words: broiler, intermittent cold stimulation, meat quality, liver lipid metabolism, AMPK-mTOR pathway

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INTRODUCTION

Chicken meat contains lower fat content and increased proportion of polyunsaturated fatty acids (PUFA) compared to meat from other species (Riovanto et al., 2012), which makes it a healthier option. Therefore, chicken meat is considered a better choice for a healthy diet. Due to the increase in consumer demand for high-quality meat products, the factors that impact the quality of chicken meat have gained significant attention from both consumers and researchers. The quality of chicken meat is influenced by various potential internal and external factors (Hussnain et al., 2020; Al-Sagan et al., 2021; Weimer et al., 2022). Temperature changes have been considered as inevitable external factors that affect the taste and quality of chicken meat. Studies have demonstrated that extremely high or low temperatures could lead to heat or cold stress, thereby

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affecting the taste and quality of chicken meat (Henrikson et al., 2018; Wen et al., 2019; Zhao et al., 2022; Maynard et al., 2023). However, Deaton et al. (1996) found that appropriate low temperature can improve the quality of chicken meat. Our previous research has indicated that the appropriate cold stimulation training can trigger cold adaptation in broilers, leading to a stronger anti-stress response under acute cold stress (Fu et al., 2022). However, up until now, there have been few reports on the effects of intermittent cold stimulation on chicken meat quality (Dadgar et al., 2012; Nyuiadzi et al., 2020).

When animals are exposed to extremely cold environments, it results in an increase in feed conversion ratio and a decrease weight gain (Zhou et al., 2021), which undoubtedly significantly increases the cost of animal feeding. Studies have indicated that prolonged cold stress or low temperatures (such as 10℃) can significantly reduce weight gain and increase the feed conversion ratio and daily feed intake for broilers (Sagher, 1975; Su et al., 2020; Zhou et al., 2021). This means that the body will inevitably undergo energy redistribution when animals are exposed to cold environments. Lipids function as high-energy organic molecules that play a crucial role in regulating the body's temperature by regulating their synthesis and decomposition. In poultry, the liver controls lipid metabolism and plays a vital role in regulating energy distribution across the body (Nguyen et al., 2008). Grefhorst et al. (2018) has reported that the exposure of animals to 4℃ for 24 h results in an increase in the content of triglycerides (**TG**) and bile acid (**BA**) in the liver, but has no effect on the level of cholesterol (**TC**), indicating that the cold can alter the liver's lipid metabolism process.

Adenosine monophosphate activated protein kinase (**AMPK**) is the main sensor of cellular energy status and is activated when the ratio of AMP to ATP increases, restoring energy balance by inhibiting ATP consumption and promoting ATP production (Hu et al., 2019). AMPK plays a vital regulatory role in lipid metabolism by phoshorylating Sterol Regulatory Element Binding Protein 1 (**SREBP1**), leading to inhibited transcriptional activity. As a result, fatty acid synthase (**FAS**), stearoyl-CoA desaturase 1 (**SCD1**), and acetyl-CoA carboxylase (**ACC**), the genes regulated by SREBP1, are downregulated (Li et al., 2011). In addition, AMPK activation can reduce the levels of acetyl-CoA, a precursor of fatty acid synthesis and an effective inhibitor of carnitine palmitoyltransferase 1 (**CPT1**), by causing phosphorylation of ACC, thus increasing CPT1 activity and leading to a decrease in fat synthesis and an increase in mitochondrial fatty acid oxidation (Kim et al., 2019). In mammals, AMPK activation can promote liver fatty acid oxidation, reduce the synthesis of TC and TG, and reduce de novo fatty acid synthesis (Winder and Hardie, 1999). Cold exposure is a related factor because studies show an increase in energy expenditure and AMPK phosphorylation levels (Wu et al., 2022).

Endoplasmic reticulum (**ER**) is the main site for liver lipid metabolism. Appropriate ER stress (**ERS**)-induced unfolded protein response (**UPR**) can maintain lipid homeostasis. However constant ERS can destroy cell function and cause apoptosis (Malhi and Kaufman, 2011). Research has shown that ERS can increase the synthesis of liver fat, inhibit the assembly and secretion of very low-density lipoprotein (**VLDL**), induce VLDL receptor expression, and promote insulin resistance, while the activation of AMPK can inhibit lipid-induced ERS and accumulation of TG (Lebeaupin et al., 2018). Autophagy is a type of catabolic process that can clear dysfunctional macromolecules and organelles via the lysosome, thus playing a vital role in preserving lipid homeostasis (Ma et al., 2021). Several studies have indicated that AMPK activation can positively regulate autophagy by inhibiting rapamycin (mTOR), thereby inhibiting the accumulation of lipids in the liver. ERS plays a significant role in regulating autophagy in cells, while autophagy contributes to removing misfolded proteins caused by ERS (Miyagawa et al., 2016). Therefore, we hypothesize that intermittent cold stimulation may alter the gene expression of lipid metabolism, ERS and autophagy in the liver of broilers, and chicken meat quality can be affected by this regulation of lipid metabolism.

To investigate how intermittent cold stimulation affects chicken meat quality, we measured the color, drip loss, shearing force, and pH value, conclusively assessing the impact of intermittent cold stimulation. Additionally, to study the effects of intermittent cold stimulation on chicken lipid metabolism, we analyzed the composition of lipids in chicken serum, assessed the expression levels of genes related to lipid metabolism, endoplasmic reticulum stress and autophagy in the liver.

MATERIALS AND METHODS

Animals and study design

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University, Harbin, China (protocol number: IACUCNEAU20150616). The experimental temperature conditions are shown in Figure 1.

One hundred sixty healthy one-day-old male Ross 308 chicks were randomly divided into 2 groups: CC group (temperature management according to the standard for broilers growth stages) and CS5 group (3℃ lower than CC group, cold stimulation for 5 h). Each group had 5 replications, and each replication had 16 broilers. Cold stimulation training was performed during the period from 15 to 35 d of age, starting at 9:30 am, every 2 d. During the 36 to 49 d of age, the temperature of all groups was maintained at 20℃. At the age of 50 d, the temperature of all groups of broilers was reduced to 10℃, and acute cold stress lasted for 24 h.

The entire experiment was performed in 2 artificial climate rooms with a stocking density of 12 broilers/m^2. The relative humidity was maintained at 60 to 70% for

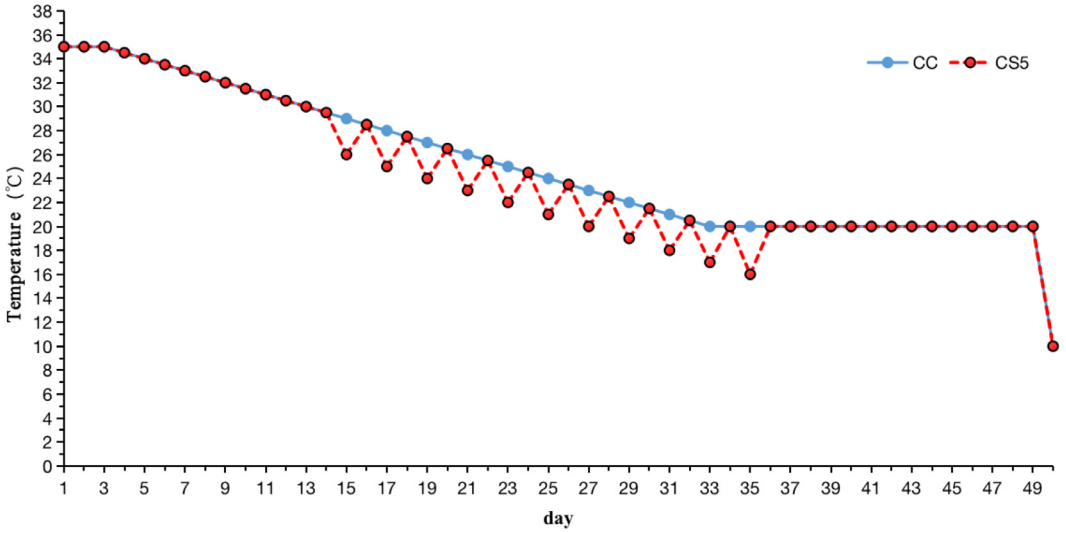

Figure 1. The specific experimental temperature scheme.

1 to 14 d and at 40 to 50% for 15 to 50 d. Lighting conditions were 24 h of light: 0 h of dark (24 L: 0 D) on 1-3 d and 23 L: 1 D on 4-50 d, and the dark time was set at 20:00-21:00 every day. The light intensity was 40 lux from 1 to 14 d and 15 lux from 15 to 50 d. The broilers were allowed to feed and drink freely. From 1 to 21 d, the broilers were fed the complete starter diet (21.00% crude protein [**CP**], 12.10 megajoules [**MJ**]/kg metabolizable energy [**ME**]), and from 22 to 50 d, the broilers were fed grower diet (19.00% CP, 12.60 MJ/kg of ME). The immunization program was executed according to the Ross broiler production standards.

Sample collection

One broiler was randomly selected from 5 replicates per group on d 36 (**36D**) and d 50 (**50D**), as well as after acute cold stress for 24 h (Y24) and slaughtering at 8:00 am. Collected blood samples were allowed to stand at room temperature for 2 h, then centrifuged at 3,000 rpm for 10 min, and the serum was collected and stored at −80℃ for subsequent ELISA assay. The liver was rapidly collected and placed in liquid nitrogen for quick freezing. Samples were stored at −80℃ for subsequent RT-PCR and Western blot analysis. Chicken breast meat was collected from 50 d chickens to assess meat quality.

Growth performance

Following the commencement of cold stimulation (15 d), all broilers in each replication group were weighed at 7:00 am on the first day of each week. Weekly feed consumption (**WFC**) was recorded. The average weight gain (**AWG**) and feed conversion ratio (**FCR**) were calculated.

$$\text{Average weight gain (g)} = (\text{Final weight} - \text{Initial weight})/\text{number of broilers per cage}$$

$$\text{Feed conversion ratio (g/g)} = \text{Weekly feed intake}/\text{Average weight gain}$$

Meat quality

pH value The breast muscle was taken from broilers 45 min after slaughter for 45 min-pH determination, which was denoted as pH1. The probe was directly inserted into the muscle tissue during the measurement and recorded after the value stabilized. After the determination of pH1, the meat sample was wrapped with plastic wrap and put it into a 4℃ refrigerator. After 24 h, the meat sample was taken out for the determination of 24 h pH value, which was recorded as pH2. The pH meter FE28-Standard (Mettler Toledo, China) was calibrated with standard solutions at pH = 4.003, pH = 6.864, and pH = 9.182 before use.

Meat color The CR-400 colorimeter (KonicaMinolta, Japan) was used to determine the color of meat samples 1 h after slaughter. The thickness of the meat sample shall not be less than 1.5 cm, and use the colorimeter to measure the L*, a* and b* value of the meat sample. The meat color values of each sample were measured in 3 different areas of the left pectoralis minor muscle, and then the average value was taken.

Drop loss A chicken breast was cut into 5 cm × 3 cm × 3 cm pieces of meat, weighed and recorded as W1. The meat was suspended in a 50 mL centrifuge tube with a string, and the centrifuge tube was placed in a refrigerator at 4℃ for 24 h. After 24 h, gently wipe the juice from the meat surface with clean filter paper, and then weigh and record as W2. Drip loss is calculated according to the formula.

$$\text{Drop loss} = (W1 - W2)/W1$$

Shearing force The meat sample after drip loss measurement was placed in a food bag, and was heated in a water bath at about 80℃ until the central temperature of the meat reached 75℃ and kept for 10 min. After removal,

a cylinder with length of 2.5 cm and diameter of 1.0 cm with scissors and placed on C-LM3B tenderness meter (TENOVO, China) for cutting. The maximum shearing force was recorded, denoted by Newton (N). Each meat sample was cut 3 times, and the average value was taken.

Oxidation and antioxidant indexes

The contents of T-AOC, SOD, GSH-Px and MDA in serum of broilers at 36D, 50D and Y24 were detected respectively. All the kits were purchased from Nanjing Jiancheng Bioengineering Research Institute, and the determination methods of the above indexes were strictly in accordance with the instructions provided by the manufacturer. The following kit models were used: T-AOC (A015-2-1), SOD (A001-3-2), GSH-Px (A005-1-2), MDA (A003-1-2).

ELISA

The serum samples from each group of chickens at 36D, 50D, and Y24 were assessed for the levels of free fatty acids (**FFA**), triglycerides (**TG**), total cholesterol (**TC**), high-density lipoprotein (**HDL**), low-density lipoprotein (**LDL**), and very low-density lipoprotein (**VLDL**) using enzyme-linked immunosorbent assay (**ELISA**) kits that were acquired from Shanghai Enzyme-Linked Biotechnology Co., Ltd. The following kits models were used: FFA (BC0595), TC (E1015), TG (E1013), HDL (ab65391), LDL (ab65390), and VLDL (ab65395). All operations were strictly performed following the instructions provided by the manufacturer.

RNA extraction and gene expression analysis

Total RNA was extracted from the chicken liver tissue using TRIzol (Takara, Dalian, China), as per the manufacturer's instructions. The dried total RNA was dissolved in 50 μL of DEPC water and then evaluated for integrity using 1.5% agarose gel electrophoresis. The purity of the RNA was assessed based on the OD260/OD280 ratio measured with an ultra-microspectrophotometer (Thermo Fisher Scientific, Carlsbad, CA). Using the ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan), complementary DNA (cDNA) was reverse-transcribed. The obtained cDNA was stored at −80℃ and duly diluted before usage for qRT-PCR.

The primer sequences applied in the experiment are provided in Table 1. These primers were designed with Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA) and synthesized by Sangon (Shanghai, China), a biotechnology firm. The LightCycler 480 II Real-Time PCR system (Roche, Basel, Switzerland) was used for qPCR amplification. The reaction system comprised 10 μL, including 1 μL of diluted cDNA, 0.3 μL of each primer, 5 μL of THUNDERBIRD SYBR qPCR Mix (Toyobo, Osaka, Japan), and 3.4 μL of DEPC water. The 3-step method was employed for PCR amplification, consisting of denaturation at 90℃ for 60 s, followed by 40 cycles of amplification at 95℃ for 15 s and annealing at 60℃ for 60 s. Melting curve analysis was carried out after each PCR. The internal reference gene was β-actin, and the relative mRNA expression level of the target gene was determined via the $2^{-\Delta\Delta Ct}$ method.

Table 1. The primers used in the experiment.

Gene	Serial number	Primer sequences (5′- 3′)
AMPK	NM_001039603.1	Forward:CGGAGATAAACAGAAGCACGAG Reverse:CGATTCAGGATCTTCACTGCAAC
mTOR	NC_006108.5	Forward:GAAGTCCTGCGCGAGCATAAG Reverse:TTTGTGTCCATCAGCCTCCAGT
SREBP1c	AY029224	Forward:GCCCTCTGTGCCTTTGTCTTC Reverse:ACTCAGCCATGATGCTTCTTCC
FAS	NM_205155.2	Forward:CAATGGACTTCATGCCTCGGT Reverse:GCTGGGTACTGGAAGACAAACA
ACC	NM_205505.1	Forward:ATGAATGGGTACTGCCTGCC Reverse:CCGTCCAGAAACACTGGTCA
SCD	NM_204,890.1	Forward:GGCTGACAAAGTGGTGATG Reverse:GGATGGCTGGAATGAAGA
PPAR	NM_001001464.1	Forward:ACCTTGTGCATGGCAGAGAA Reverse:TACACTGGCAGCAGTGGAAG
CPT	XM_025150696.1	Forward:TGGCTGATGATGGTTACGGTG Reverse:TTCCAAAGCGATGAGAATCCGTT
Beclin1	NC_052558.1	Forward:GAGAGTCAGGGCAGAGGCAGAG Reverse:ACACAGTCCAAGAAAGCCACCATC
PERK	NC_052535.1	Forward:GGGCGAGGATGTTGTCTTAGTTGG Reverse:GCCGAGCAGATGTACTTCACCTTC
GRP78	NC_052548.1	Forward:CCTCCTGCTCCTCGTGGTGTC Reverse:TCTCCTCTGGTGTTAGCCGATTCTG
CHOP	NC_052606.1	Forward:CAGTGTGCTGTGAGCTGGATGAG Reverse:CACGCTTCCGCTTTGTCCTCTG
LC3	XM_417327.7	Forward:CCTGGTGCCAGATCACGTCAAC Reverse:AAGCCGTCCTCGTCCTTCTCG
β-actin	NM_205518.1	Forward:CACCACAGCCGAGAGAGAAAT Reverse:TGACCATCAGGGAGTTCATAGC

Western blot analysis

Total protein was extracted from frozen broiler liver tissue using Western IP cell lysis buffer (SparkJade, Harbin, China) containing 1% phenylmethylsulfonyl fluoride (PMSF) (Biosharp, Beijing, China). We measured the protein concentration and adjusted it to 4 μg/μL using the BCA protein concentration determination kit (SparkJade, Harbin, China). Equal amounts of total protein (40 μg per condition) were subjected to SDS-PAGE. The protein was then transferred onto an NC membrane (SparkJade, Harbin, China) using semi-dry transfer equipment. The membrane was blocked at 37℃ for 2 h using 5% skim milk and washed 3 times with phosphate-buffered saline with Tween (PBST). We then incubated the NC membrane overnight at 4℃ with specific primary antibodies, including: AMPK (1:500, Wanleibio, Shenyang, China); p-AMPK (1:500, Wanleibio, Shenyang, China); GRP78 (1:1000, Wanleibio, Shenyang, China); LC3 (1:600, Wanleibio, Shenyang, China); ATG7 (1:500, Wanleibio, Shenyang, China); and β-actin (1:9000, ABclonal, Harbin, China). We washed the membrane with PBST 3 times before incubating it with HRP-labeled goat anti-rabbit IgG (1:9000, ABclonal, Harbin, China) for 2 h. Lastly, we used an ELC chemiluminescent detection kit (SparkJade, Harbin, China) and a grayscale scanner to observe the protein bands. We analyzed the resulting images using ImageJ software (NIH, Bethesda, MD) and calculated the ratio of the grayscale value for each target protein to that of β-actin to represent the relative expression of the target protein.

Statistical analysis

Data were analyzed using SPSS 21.0 (IBM, Armonk, NY), with Levene's test used to assess the homogeneity of variance between the 2 groups. Subsequently, independent sample t-test was conducted to evaluate the impact of intermittent cold stimulation (ICS) on various factors including growth performance, meat quality, antioxidant capacity, mRNA and protein expression levels of liver genes, and serum lipid composition. All figures were created using GraphPad Prism 9.0 for Mac (GraphPad Software, San Diego, CA). The results are presented as the mean ± standard error of the mean (SEM), with statistical significance indicated by P values less than and equal to 0.05.

RESULTS

Effect of cold stimulation on the growth performance of broilers

To investigate the macroscopic effects of intermittent cold stimulation on broilers, we examined the related indicators of broiler growth performance. As shown in Table 2, the average weight gain of CS5 group broilers was significantly higher than that of CC group at 4 W and 6 W ($P_{(4W)} = 0.017$; $P_{(6W)} = 0.007$). There was no significant difference in weekly feed intake between CC and CS5 group broilers ($P_{(3W)} = 0.871$; $P_{(4W)} = 0.984$; $P_{(5W)} = 0.353$; $P_{(6W)} = 0.579$; $P_{(7W)} = 0.561$). At 4 W and 6 W, the feed conversion ratio of CS5 group broilers was significantly lower than that of CC group ($P_{(4W)} = 0.001$; $P_{(6W)} = 0.047$).

Effect of cold stimulation on meat quality of broilers

As shown in Figures 2A and 2B, the drip loss and shearing force of CS5 group broiler breast meat were significantly lower than those of CC group ($P_{(\text{drip loss})} = 0.009$; $P_{(\text{shearing force})} = 0.001$). As shown in Figure 2C, there was no significant difference in pH1 and pH2 values between CC and CS5 group broilers ($P_{(\text{pH1})} = 0.052$; $P_{(\text{pH2})} = 0.141$). As shown in Figures 2D–2F, there was no significant difference in L* value between the 2 groups ($P = 0.449$), the a* value of CC group was significantly higher than that of CS5 group ($P = 0.013$), while the b* value of CS5 group was significantly higher than that of CC group ($P = 0.001$).

Effect of cold stimulation on the antioxidant capacity of broilers

As shown in Figure 3, at 36D, the level of T-AOC in the serum of CS5 group broilers was significantly higher than that of CC group ($P = 0.041$). The levels of SOD,

Table 2. Effect of intermittent cold stimulation on growth performance of Ross 308 broilers.

Week	Group	AWG (g/broiler/W)	P value	WFC (g/broiler/W)	P value	FCR (g/g)	P value
3W	CC	453.13 ± 8.09	0.848	681.25 ± 13.11	0.871	1.45 ± 0.01	0.408
	CS5	447.50 ± 27.20		683.75 ± 7.15		1.42 ± 0.03	
4W	CC	400.00 ± 10.21[a]	0.017	961.33 ± 24.80	0.984	2.40 ± 0.06[b]	0.001
	CS5	568.75 ± 37.33[b]		962.00 ± 20.10		1.63 ± 0.07[a]	
5W	CC	687.50 ± 16.30	0.644	1,255.71 ± 37.74	0.353	1.83 ± 0.06	0.150
	CS5	662.50 ± 45.07		1,310.72 ± 40.99		2.04 ± 0.10	
6W	CC	734.38 ± 33.99[a]	0.007	1,550.77 ± 51.25	0.579	1.96 ± 0.02[b]	0.047
	CS5	916.67 ± 23.20[b]		1,512.31 ± 42.24		1.85 ± 0.19[a]	
7W	CC	540.63 ± 44.30	0.376	1,520.83 ± 40.43	0.561	2.58 ± 0.17	0.119
	CS5	612.50 ± 57.28		1,485.83 ± 40.71		2.25 ± 0.00	

Note: Data were presented as mean ± standard error of the mean.
[a,b]Different letters indicate significant differences ($P \leq 0.05$) between treatment groups (a, b).Abbreviations: AWG, average weight gain; WFC, weekly feed consumption; FCR, feed conversion ratio.

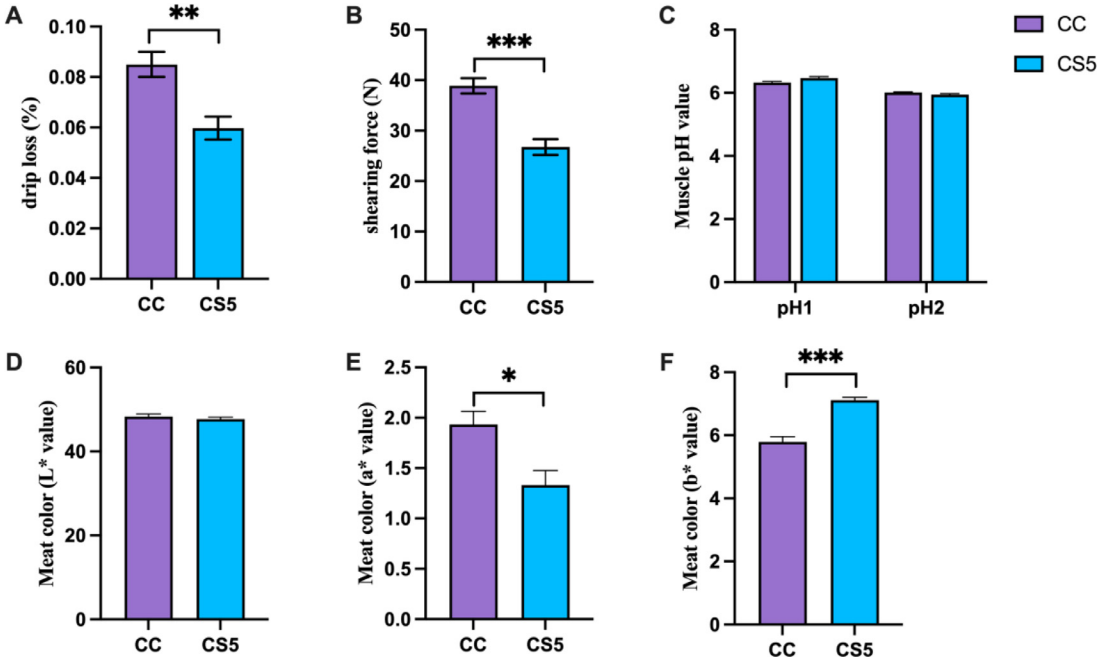

Figure 2. Effect of intermittent cold stimulation on meat quality of broilers. Drip loss (A), Shearing force (B), pH value (C), Meat color L* value (D), Meat color a* value (E), Meat color b* value (F). Data were presented as mean ± standard error of the mean. (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

GSH-Px and MDA in the CS5 group were higher than those in the CC group, but the difference was not significant ($P_{(SOD)} = 0.860$; $P_{(GSH\text{-}Px)} = 0.295$; $P_{(MDA)} = 0.364$). At 50D, the serum GSH-Px level in the CS5 group was significantly higher than that in the CC group ($P = 0.001$). There was no significant difference in T-AOC, SOD and MDA levels between the 2 groups ($P_{(T\text{-}AOC)} = 0.696$; $P_{(SOD)} = 0.453$; $P_{(MDA)} = 0.981$). At Y24, the serum T-AOC and GSH-Px levels of CS5 group broilers were significantly higher than those of CC group ($P_{(T\text{-}AOC)} = 0.001$, $P_{(GSH\text{-}Px)} = 0.001$), while the MDA level of CC group was significantly higher than that of CS5 group ($P = 0.043$). The SOD level of CS5 group was higher than that of CC group, but the difference was not significant ($P = 0.642$).

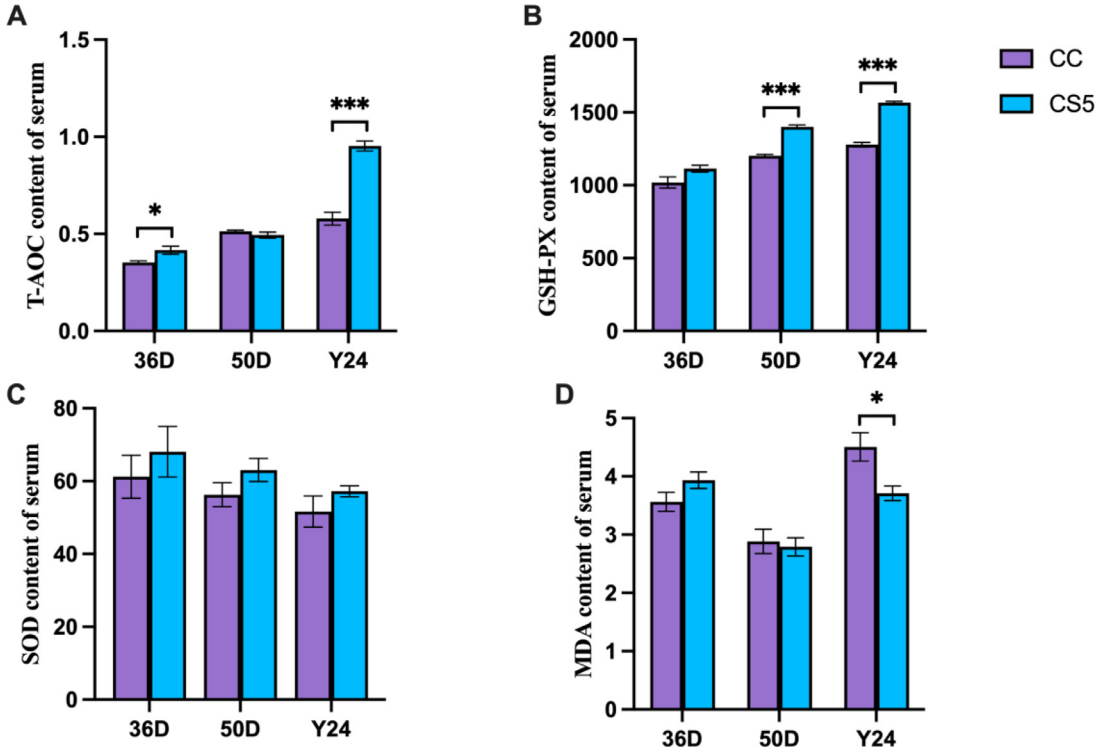

Figure 3. Effect of cold stimulation on antioxidant capacity of broilers. T-AOC (A), GSH-Px (B), SOD (C) and MDA (D). Data were presented as mean ± standard error of the mean. (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

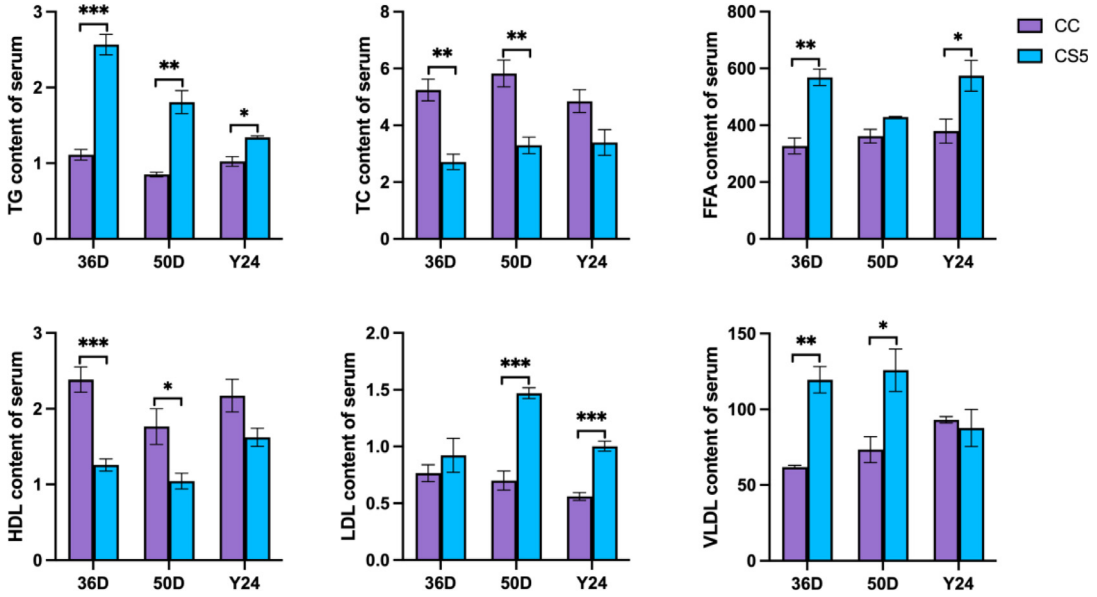

Figure 4. The effect of cold stimulation on the serum lipid composition of broilers. TG (A), TC (B), FFA (C), HDL (D), LDL (E) and VLDL (F). Data were presented as mean ± standard error of the mean. (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

Effect of cold stimulation on the serum lipid composition of broilers

To investigate the impact of intermittent cold stimulation on lipid metabolism in broilers, we first tested their serum lipid composition (Figure 4). At 36D, this results showed that intermittent cold stimulation significantly increased TG, FFA, and VLDL in serum ($P_{(\mathrm{TG})} = 0.001$, $P_{(\mathrm{FFA})} = 0.006$, $P_{(\mathrm{VLDL})} = 0.006$), while significantly decreasing TC and HDL ($P_{(\mathrm{TC})} = 0.002$, $P_{(\mathrm{HDL})} = 0.001$). At 50D, the CS5 group had significantly higher serum content of TG, LDL, and VLDL than the CC group ($P_{(\mathrm{TG})} = 0.003$, $P_{(\mathrm{LDL})} = 0.001$, $P_{(\mathrm{VLDL})} = 0.024$), while serum content of TC and HDL was significantly lower ($P_{(\mathrm{TC})} = 0.006$, $P_{(\mathrm{HDL})} = 0.048$). Finally, at Y24, the contents of TG, FFA, and LDL in the CS5 group were significantly higher than those in the CC group ($P_{(\mathrm{TG})} = 0.029$, $P_{(\mathrm{FFA})} = 0.047$, $P_{(\mathrm{LDL})} = 0.001$). Although the CC group had higher TC and HDL levels, the difference was no significant ($P_{(\mathrm{TC})} = 0.175$, $P_{(\mathrm{HDL})} = 0.290$).

Effect of cold stimulation on the expression of AMPK and mTOR genes in the liver of broilers

As a sensor of cellular energy, AMPK can be activated to co-regulate the metabolism of multiple organs by inhibiting mTOR expression. In order to investigate the effect of intermittent cold stimulation on the expression of *AMPK* and *mTOR* genes, we detected the mRNA expression levels (Figure 5A and B). The results showed that, at 36D, the mRNA expression level of *AMPK* in the liver of broilers in the CS5 group was significantly

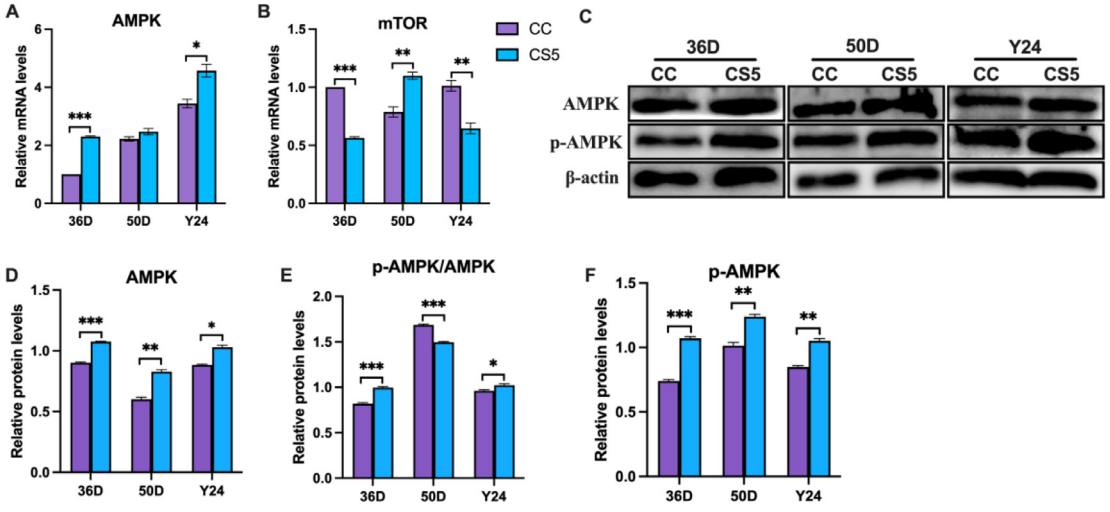

Figure 5. The effect of cold stimulation on the gene expression of AMPK and mTOR in broilers. AMPK mRNA (A), mTOR mRNA (B), Protein bands (C), AMPK protein (D) and p-AMPK protein (E), p-AMPK/AMPK (F). Data were presented as mean ± standard error of the mean. (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

higher than that in the CC group ($P = 0.001$), while the mRNA expression level of *mTOR* was significantly lower than that in the CC group ($P = 0.001$). At 50D, there was no significant difference in the mRNA expression level of *AMPK* between the 2 groups ($P = 0.348$), while the mRNA expression level of mTOR in the liver of broilers in the CS5 group was significantly higher than that in the CC group ($P = 0.006$). Finally, at Y24, the mRNA expression level of *AMPK* in the liver of broilers in the CS5 group was significantly higher than that in the CC group ($P = 0.016$), while the mRNA expression level of mTOR was significantly lower than that in the CC group ($P = 0.005$). Subsequently, we examined the expression levels of AMPK protein (Figure 5C–F). The results indicated that the protein expressions of phosphorylated and non-phosphorylated AMPK in the liver of broilers in the CS5 group were constantly and significantly higher than those in the CC group at each time point (36D: $P_{(\text{AMPK})} = 0.001$, $P_{(\text{p-AMPK})} = 0.001$; 50D: $P_{(\text{AMPK})} = 0.002$, $P_{(\text{p-AMPK})} = 0.009$; Y24: $P_{(\text{AMPK})} = 0.023$, $P_{(\text{p-AMPK})} = 0.004$). By calculating the ratio of phosphorylated to non-phosphorylated AMPK, it was revealed that, at 36D and Y24, the ratio of the CS5 group was significantly higher than that of the CC group ($P_{(36\text{D})} = 0.000$, $P_{(\text{Y24})} = 0.032$). However, at 50D, the ratio of the CC group was significantly higher than the CS5 group ($P = 0.001$).

Effect of cold stimulation on mRNA expression levels of lipid metabolism-related genes in the liver of broilers

AMPK can phosphorylate SREBP1c, inhibit its transcriptional activity, and downregulate lipid synthesis-related genes regulated by SREBP1c. In addition, AMPK activation can promote hepatic fatty acid oxidation. Therefore, we investigated the effect of intermittent cold stimulation on the expression of lipid synthesis and degradation genes in the liver of broilers (Figure 6). The results showed that at 36D, compared with the CC group, the mRNA expression levels of *SREBP1c, FAS*, *SCD*, and *ACC*, which are genes related to lipid synthesis, were significantly decreased in the CS5 group ($P_{(\text{SREBP1c})} = 0.001$, $P_{(\text{FAS})} = 0.008$, $P_{(\text{SCD})} = 0.001$, $P_{(\text{ACC})} = 0.001$). The mRNA expression levels of *PPAR* and *CPT* genes in the CS5 group were significantly higher than those in the CC group ($P_{(\text{PPAR})} = 0.001$, $P_{(\text{CPT})} = 0.015$). At 50D, the mRNA expression levels of *SREBP1c, SCD*, and *ACC* in the CC group were significantly higher than those in the CS5 group ($P_{(\text{SREBP1c})} = 0.044$, $P_{(\text{SCD})} = 0.001$, $P_{(\text{ACC})} = 0.001$). The mRNA expression levels of FAS in the CC group were higher than those in the CS5 group, but the difference was not significant ($P = 0.297$). The mRNA expression levels of *PPAR* and *CPT* in the CS5 group were significantly higher than those in the CC group ($P_{(\text{PPAR})} = 0.001$, $P_{(\text{CPT})} = 0.001$). Finally, at Y24, the mRNA expression levels of *SREBP1c, FAS, SCD, ACC*, and *CPT* in the CC group were significantly higher than those in the CS5 group ($P_{(\text{SREBP1c})} = 0.001$, $P_{(\text{FAS})} = 0.010$, $P_{(\text{SCD})} = 0.046$, $P_{(\text{ACC})} = 0.049$, $P_{(\text{CPT})} = 0.008$). The mRNA expression levels of the *PPAR* gene in the CS5 group were significantly higher than those in the CC group ($P = 0.049$), while the mRNA expression levels of the *CPT* gene were significantly lower than those in the CC group ($P = 0.008$).

Effect of cold stimulation on the expression of endoplasmic reticulum stress-related genes in the liver of broilers

The endoplasmic reticulum (**ER**) is an important site for liver lipid metabolism, and appropriate endoplasmic

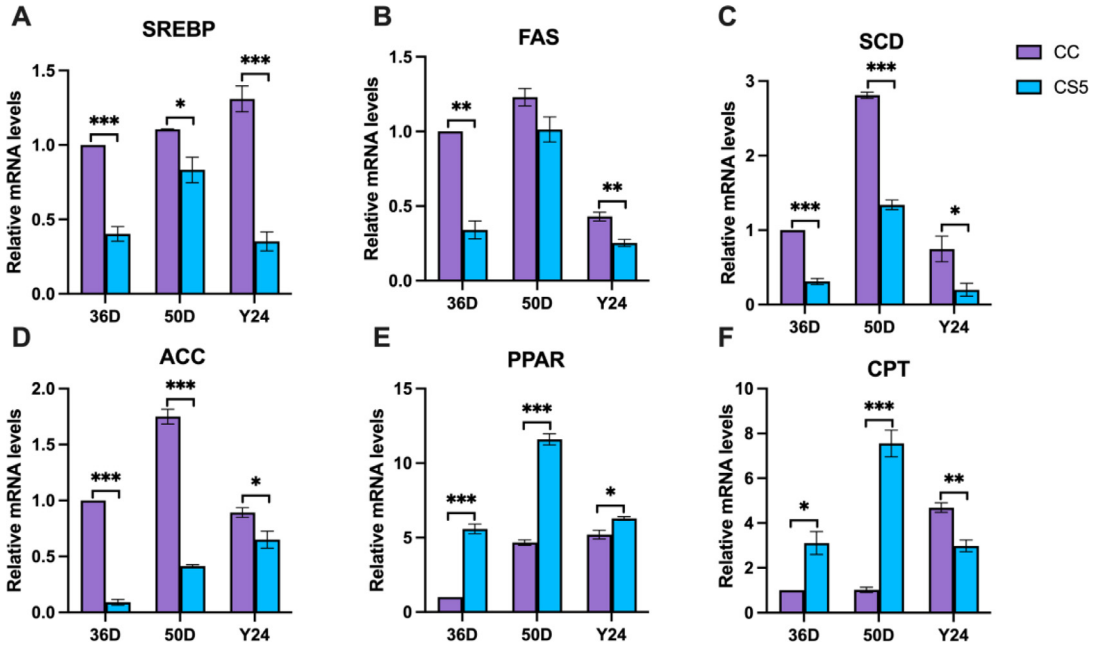

Figure 6. The effect of cold stimulation on the mRNA expression levels of liver lipid metabolism-related genes in broilers. SREBP1c (A), FAS (B), SCD (C), ACC (D), PPAR (E) and CPT (F). Data were presented as mean ± standard error of the mean. (*$P \le 0.05$, **$P \le 0.01$, ***$P \le 0.001$).

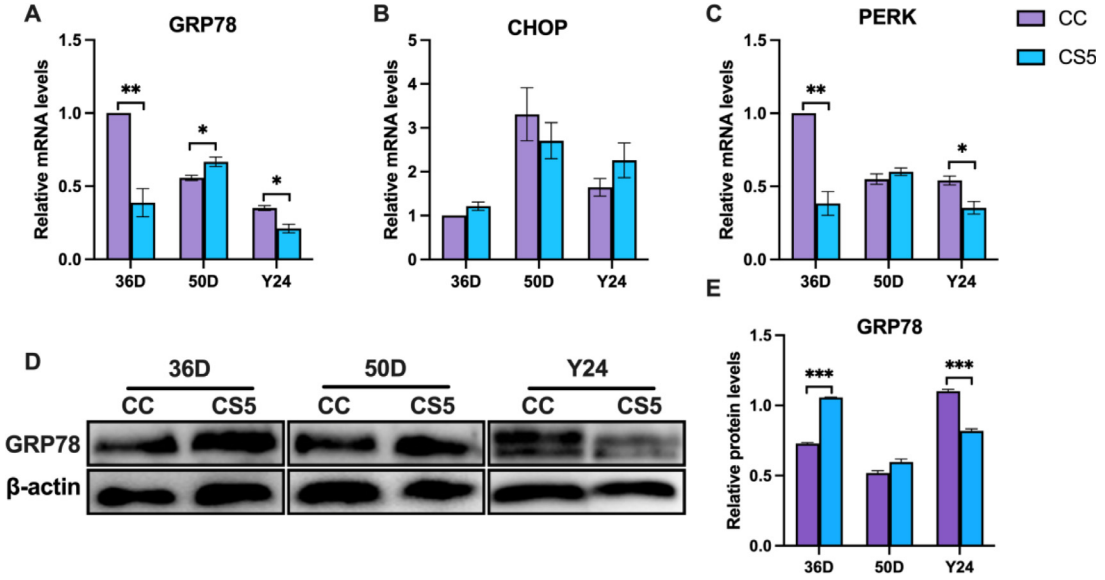

Figure 7. The effect of cold stimulation on the expression of endoplasmic reticulum stress-related genes in the liver of broilers. GRP78 mRNA (A), CHOP mRNA (B), PERK mRNA (C), Protein bands (D) and GRP78 protein (E). Data were presented as mean ± standard error of the mean. (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

reticulum stress (**ERS**) can maintain lipid homeostasis. In order to investigate the effect of intermittent cold stimulation on ERS in the liver of broilers, we detected the mRNA expression levels of related genes (Figure 7A−C). The results showed that, at 36D, the mRNA expression levels of *GRP78* and *PERK* genes in the liver of broilers in the CC group were significantly higher than those in the CS5 group ($P_{(GRP78)} = 0.024$, $P_{(PERK)} = 0.017$). At 50D, the mRNA expression level of *GRP78* gene in the CS5 group was significantly higher than that in the CC group ($P = 0.042$), while there was no significant difference in *PERK* gene between the CC and CS5 groups ($P = 0.682$). At Y24, the mRNA expression levels of *GRP78* and *PERK* genes in the CC group were significantly higher than those in the CS5 group ($P_{(GRP78)} = 0.021$, $P_{(PERK)} = 0.029$). There was no significant difference in *CHOP* gene between the 2 groups at each time point (36D: $P = 0.603$; 50D: $P = 0.843$; Y24: $P = 0.594$). Then, to further verify the effect of intermittent cold stimulation on ERS, we detected the protein level of GRP78 (Figure 7D and E). The results showed that intermittent cold stimulation significantly increased the protein expression level of GRP78 at 36D ($P = 0.001$). At 50D, there was no significant difference in the protein expression level of GRP78 between the CC and CS5 groups ($P = 0.151$). At Y24, the protein expression level of GRP78 in the CS5 group was significantly lower than that in the CC group ($P = 0.001$).

Effect of cold stimulation on the expression of autophagy-related genes in the liver of broilers

Activation of AMPK induces autophagy, which plays a crucial role in maintaining lipid metabolism homeostasis. Therefore, to explore the role of intermittent cold stimulation in inducing autophagy in the liver cells of broilers, we first detected the mRNA expression levels of autophagy-related genes (Figure 8A and B). The results showed that, except for the significantly lower mRNA expression level of *Beclin1* gene in the CS5 group than that in the CC group at 36D ($P = 0.011$), the mRNA expression levels of *LC3* and *Beclin1* in the CS5 group were significantly higher than those in the CC group at all other time points (36D: $P_{(LC3)} = 0.048$; 50D: $P_{(LC3)} = 0.005$, $P_{(Beclin1)} = 0.003$; Y24: $P_{(LC3)} = 0.003$, $P_{(Beclin1)} = 0.001$). To further explore the effect of cold stimulation on autophagy, we detected the protein expression levels of autophagy-related genes (Figure 8C−E). The results showed that, except for the significantly lower protein expression level of ATG7 in the CS5 group than that in the CC group at 50D ($P = 0.015$), the protein expression levels of LC3 and ATG7 in the CS5 group were significantly higher than those in the CC group at all other time points (36D: $P_{(LC3)} = 0.001$, $P_{(ATG7)} = 0.002$; 50D: $P_{(LC3)} = 0.002$; Y24: $P_{(LC3)} = 0.001$, $P_{(ATG7)} = 0.002$).

DISCUSSION

Low temperature has been widely concerned as a main factor affecting the development of poultry industry in northern China. Previous research in our laboratory has shown that appropriate cold stimulation training can improve the productivity and cold stress resistance of broilers. However, studies such as Mendes et al. (1997) have indicated that 15℃ cold exposure significantly increased broiler feed intake, feed conversion ratio, and mortality in broilers aged 21 to 42 d, as well as significantly decreased body weight of broilers at 42 d. In contrast, Su et al. (2020) study showed that rearing broilers 3℃ lower than the conventional temperature had no

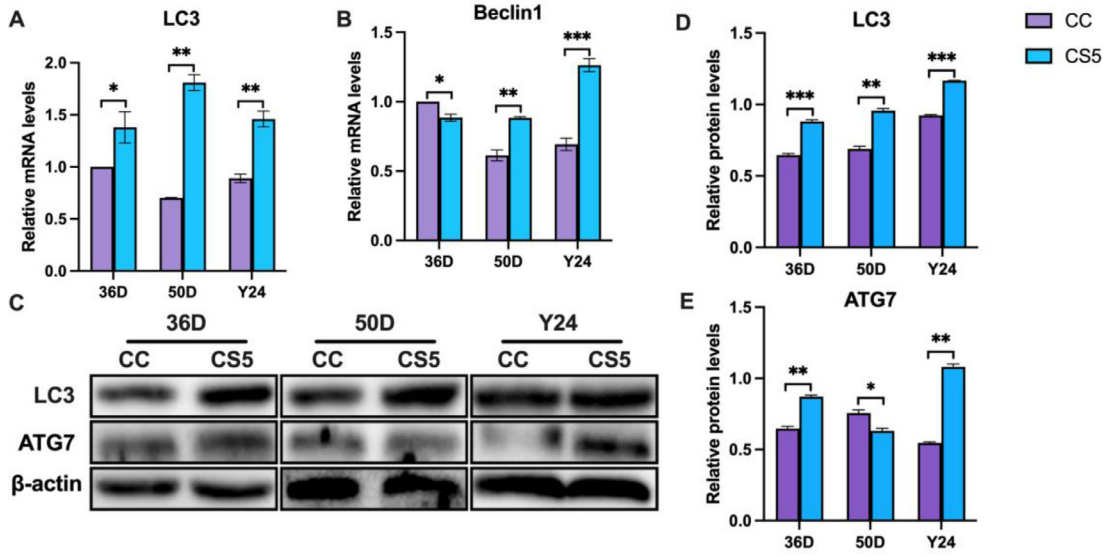

Figure 8. The effect of cold stimulation on the expression of autophagy-related genes in the liver of broilers. LC3 mRNA (A), Beclin1 mRNA (B), Protein bands (C), LC3 protein (D) and ATG7 protein (E). Data were presented as mean ± standard error of the mean. (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$).

adverse effect on the productivity of broilers, and the feed conversion ratio and daily weight gain were better than those of conventionally reared broilers in the 4 W and 5 W, respectively. The results of this study showed that the average weekly weight gain of broilers in the CS5 group was significantly higher than those in the CC group at 4 W and 6 W, and the feed conversion ratio was significantly lower than those in the CC group at 4 W and 6 W. Except for 7 W, there was no significant difference in feed intake among the 2 groups, indicating that the changes in average weekly weight gain and feed conversion ratio among the 2 groups were not caused by changes in feed intake. As homothermal animal, broilers can re-establish dynamic balance of body temperature through corresponding adaptive mechanisms in cold environments. However, the establishment of this new balance changes the basal metabolic rate and energy metabolism of broilers (Chen et al., 2014). Consequently, the observed improvement in productivity of the broilers in the CS5 group may be due to altered energy metabolism of broilers resulting from intermittent cold stimulation training.

Meat quality is the result of multiple indexes, and there is a certain correlation among indexes. Drip loss is a manifestation of muscle water-holding capacity. Good water-holding capacity can better maintain the nutrition, taste, aroma and tenderness of meat, and can impact meat color to a certain extent (Luciano et al., 2009). In this study, the CS5 group has lowest drip loss and stronger water-holding capacity compared to CC group. Yang and Chen (1993) found that a decrease in pH value might be a significant factor contributing to an increase in muscle drip loss. However, in our study, there was no significant difference in pH value between the CS5 and CC groups, which indicates that the improvement in drip loss by intermittent cold stress may not be due to pH regulation. The research indicates that increased muscle oxidation results in higher water loss (Berardo et al., 2015). A study by Jin et al. (2021) revealed that resveratrol significantly enhances the antioxidant capacity of ducks, reducing the drip loss of duck meat. According to this study, intermittent cold stimulation markedly boosts the antioxidant capacity of broilers. Hence, we conclude that the decrease in drip loss of chicken meat is a result of the improved antioxidant capacity. Muscle tenderness is usually expressed by shearing force, with lower shearing force indicating greater muscle tenderness, and shearing force is usually positively correlated with drip loss. Froning et al. (1978) and Babji et al. (1982) found that exposing turkeys and chickens to moderate cold environments could make their breast meat more tender. In our study, the CS5 group had lower shearing force and more tender meat. Meat color is an important factor affecting consumers' preference for broiler meat, although there is no direct relationship between the meat color and the meat quality (Terlouw, 2005). For broiler breast meat, L* values of 42 to 50 and a* values of 1.9 to 5.3 have been widely reported (Chen et al., 2020; Chang et al., 2020; Kim et al., 2020). Nyuiadzi et al. (2020) found that, compared with the control group (0 to 21 d, 32 to 21℃), broilers reared at low temperature (0 to 21 d, 29 to 21℃) had lower a* value in breast meat. Nyuiadzi et al. (2020) thought that this may be related to changes in the metabolic rate of broilers or a shift in the metabolic type of muscle fibers to a more oxidizing metabolism. Čobanović et al. (2020) found that compared with pigs reared in autumn, the meat water-holding capacity of pigs reared in winter has lower water capacity and lighter color. Chen et al. (2023) found that the b* value of myofibrillar protein from oxidized beef decreased after MDA oxidation. In this study, cold stimulation did not significantly affect the L* value of the muscle, but significantly reduced the a* value and increased the b* value. We believe that the appearance of this phenomenon is partially caused by the enhanced antioxidant capacity of

broilers, but further exploration is still needed. In summary, intermittent cold stress improved the quality of the muscle to a certain extent.

Oxidative stress is closely related to the immune function of the body, and cold stress can cause oxidative stress by destroying the balance between the oxidation and antioxidant systems of the body, thus damaging the immune function (Wei et al., 2018). Ramnath and Rekha (2009) found that the activities of SOD and GSH-Px in serum of chickens were significantly reduced after 10 d of cold exposure at 4℃, while the content of MDA was significantly increased . The study of Zhao et al. (2014) also showed that cold stress caused the increase of MDA content in thymus of broilers. In this study, at 36D, compared with the CC group, the CS5 group had a higher level of T-AOC. At 50D, the level of GSH-Px in the CS5 group was significantly higher than that in the CC group. This indicates that intermittent cold stimulation can improve the antioxidant capacity of broilers. The above results are similar to the research findings of Wei et al. (2023), both demonstrating that cold stimulation training can enhance the antioxidant capacity of broilers. Interestingly, at Y24, the serum levels of T-AOC and GSH-Px in the CS5 group were significantly higher than those in the CC group, while the MDA level was significantly lower than that in the CC group. Generally, animals can form cold adaptation after 21 d of cold exposure in low temperature environment. Cold adaptation can enhance the cold tolerance of body, and prevent and reduce the damage caused by cold stress to a certain extent (Bukowiecki et al., 1986). AMPK serves as a pivotal energy sensor for cellular metabolism under diverse metabolic stresses and has been demonstrated to mitigate the oxidative stress response through the regulation of multiple pathways (Han et al., 2019; Ren et al., 2020; Tanaka et al., 2023). Shen et al. (2023) found that activating the AMPK/mTOR signaling pathway effectively mitigates oxidative stress damage resulting from excessive lipid accumulation, which may be the main reason why broilers in CS5 group have stronger antioxidant capacity after acute cold stress. In addition, heat stress has been found to affect pH and water-holding capacity of poultry meat by promoting glucocorticoid synthesis, enhancing corticosterone levels and ROS formation, and ultimately resulting in increased muscle glycogen content (Ma et al., 2021). Transport stress has also been shown to have negative effects on poultry meat, and these negative effects may be caused by stress reducing the antioxidant activity of poultry meat and producing more oxidizing free radicals (Pan et al., 2018). Therefore, the enhancement of antioxidant capacity may also be an important factor in improving the quality of chicken in this study.

Chickens maintain their body temperature under low-temperature conditions by regulating the breakdown and synthesis of lipids. To investigate the effects of intermittent cold stimulation on energy metabolism, the concentrations of different lipid types were monitored. TG represent an important energy storage substance that can be hydrolyzed into FFA when the body needs a large amount of energy in response to environmental demands (Nakamura et al., 2014). Research by Wang et al. have shown that acute and chronic cold stress can lower serum TG levels and increase FFA levels (Wang et al., 2009). Poursharifi et al. (2020) discovered that exposure to cold stress can stimulate lipid catabolism, elevate free fatty acid concentrations, and consequently augment heat production. In this study, intermittent cold stimulation significantly increased the content of FFA in broiler serum, and the FFA content in the CS5 group of broilers was significantly higher than that in the CC group after acute cold stress. Surprisingly, this study also revealed that intermittent cold stimulation did not reduce TG concentration in chicken serum. In fact, the concentration of TG in chicken serum of the CS5 group was higher than that in the CC group on 36D, 50D, and Y24. VLDL, predominantly composed of TG, is typically used as an indicator to assess endogenous TG (van Es, 1977; Nakamura et al., 2014). Therefore, the content of VLDL in serum shows changes similar to TG. These changes imply that under intermittent cold stimulation, the existing TG is not being converted to FFA, but rather alterations in the total quantity of TG and FFA occur. The research of Zhang et al. (2023) and Xing et al. (2023) indicates that suitable cold stress training can improve broilers' immune capacity and intestinal barrier integrity. We propose that cold stress training can enhance the relevant functions of broiler intestines. Thus, we speculate that the rise in TG levels may result from the enhanced digestive capability of broilers, which is also manifested in the reduced feed conversion ratio. LDL is responsible for conveying cholesterol all over the body, while HDL transports lipids containing TG, LDL, and TC, from the peripheral tissues to the liver (Schumaker et al., 1994). Zhang et al. (2014) found that cold stress treatment significantly changed the cholesterol composition in broiler serum, including the increase of HDL and LDL. However, our results differ slightly from theirs. Our study found that intermittent cold stimulation at 36D, 50D, and Y24 increased serum LDL concentration, while in the cold stimulation group, the serum HDL content was lower than that in the CC group at all time points. Thus, intermittent cold stimulation enhances the ability of chicken liver to transmit cholesterol to peripheral tissues but reduces its ability to send it back. In summary, intermittent cold stimulation can change the lipid components in broiler serum and can affect changes in lipid components in broiler serum during acute cold stress.

AMPK activation regulates liver lipid metabolism through multiple mechanisms (Li et al., 2014; Tamargo-Gómez and Mariño, 2018). Firstly, AMPK can inhibit lipid synthesis by inhibiting the expression of ACC and SREBP1c (Li et al., 2011; Kim et al., 2019). ACC is a fatty acid synthesis enzyme that regulates the synthesis of acetyl-CoA. AMPK activation phosphorylates ACC, leading to ACC inactivation, reducing acetyl-CoA levels, thereby inhibiting hepatic fat synthesis and stimulating fatty acid oxidation. Our results showed that the mRNA expression level of ACC in the CS5 group was

significantly lower than that in the CC group. In addition, AMPK activation also inhibits the transcriptional activity of SREBP1c via its phosphorylation. Studies have shown that phosphorylation of Ser372 in SREBP1c inhibits the protein hydrolysis and translocation activation of SREBP1, thus inhibiting the expression of SREBP1c target fat synthesis genes FAS, SCD, and ACC, and also inhibiting the expression of SREBP1c (Su et al., 2020). The results showed that except for no significant difference in FAS mRNA expression level between the CS5 group and the CC group at 50D, the mRNA expression levels of SREBP1c, FAS, and SCD genes in the cold stimulation group were significantly lower than those in the CC group at other time points. Moreover, AMPK promotes fatty acid oxidation by increasing PPARα-mediated fatty acid oxidation genes, such as CPT (Day et al., 2017). The results showed that intermittent cold stimulation significantly increased the mRNA expression level of PPAR genes in the liver, and the mRNA expression level of the CPT gene in the CS5 group was significantly higher than that in the CC group at 36D and 50D. In brief, the results show that intermittent cold stimulation reduces the expression of lipid synthesis genes in broiler liver and increases that of lipid breakdown genes. Notably, this trend continues to some extent until 2 wk later. Compared to the control group, broilers trained using intermittent cold stimulation have lower lipid synthesis gene expression levels and higher lipid breakdown gene expression levels during acute cold stress. This indicates that intermittent cold stimulation training can improve the ability of broilers to resist cold stress by regulating lipid synthesis and breakdown.

AMPK activation can also inhibit endoplasmic reticulum stress through multiple ways, thereby inhibiting hepatic lipid degeneration (Li et al., 2014; Mansour et al., 2022). Prolonged activation of endoplasmic reticulum stress increases lipid synthesis by activating SREBP1c and reducing the output of intracellular TG by VLDL, leading to hepatic fat accumulation (Lebeaupin et al., 2018). Over-nutrition activates the mTOR signaling pathway, inducing endoplasmic reticulum stress and the expression of lipid synthesis-related genes (Lu et al., 2019; Tian et al., 2023). Therefore, AMPK activation can inhibit endoplasmic reticulum stress and prevent hepatic lipid accumulation by inhibiting mTOR expression. By testing the changes in mRNA and protein expression levels of endoplasmic reticulum stress-related genes, we further validated the effect of AMPK activation on endoplasmic reticulum stress in the liver. The results showed that at 36D and Y24, the mRNA expression levels of the GRP78 and PERK genes in the CS5 group were significantly lower than those in the CC group, and the protein expression level of the GRP78 in the CS5 group was significantly lower than that in the CC group. In summary, intermittent cold stimulation can affect endoplasmic reticulum stress in broiler liver, and intermittent cold stimulation training can make broilers exhibit lower levels of endoplasmic reticulum stress when subjected to acute cold stress, especially in the CS5 group. Therefore, endoplasmic reticulum stress is involved in the regulation of broiler lipid metabolism by intermittent cold stimulation.

Finally, AMPK activation also promotes autophagy (Tamargo-Gómez and Mariño, 2018; Zhang et al., 2023). Autophagy plays an important role in intracellular lipid degradation and can utilize acidic hydrolase to degrade lipid droplets (Schulze et al., 2017; Gong et al., 2023). Inhibiting autophagy-mediated intracellular TG breakdown plays an important role in lipid accumulation in the liver. Our research found that at 36D, intermittent cold stimulation significantly increased the expression level of LC3 gene mRNA in the liver of CS5 group broilers, and this effect persisted for the second week after the end of intermittent cold stimulation. After acute cold stress, the mRNA expression levels of LC3 and Beclin1 in the CS5 group were significantly higher than those in the CC group. In addition, we also detected the protein expression of autophagy-related genes at the protein level. The results showed that after acute cold stress, the protein expression levels of LC3 and ATG7 in the liver of the CS5 group broilers were significantly higher than those in the CC group. AMPK activates lipid metabolism by inhibiting mTOR, whereas mTOR can inhibit autophagy by inhibiting the formation of autophagosomes (Kim et al., 2019; Gong et al., 2023). AMPK activation inhibits the expression of mTOR, thereby promoting cell autophagy. Studies have shown that ER stress can regulate autophagy in cells (Lavallard and Gual, 2014). Based on our detection of ER stress levels, the decrease in ER stress level mediated by intermittent cold stimulation may also help activate autophagy. In summary, autophagy is also involved in the regulation of broiler lipid metabolism by intermittent cold stimulation.

CONCLUSIONS

In this study, we found that intermittent cold stimulation can reduce the drip loss, shearing force, and a* value of chicken meat and improve its quality. It can also improve the antioxidant stress resistance of broilers. In addition, it can affect the lipid metabolism in the liver of broilers during cold stimulation and acute cold stress, and CS5 group exhibit stronger lipid decomposition ability when facing acute cold stress. This phenomenon is believed to be mediated by the activation of AMPK, which inhibits the expression of lipid synthesis genes, enhances the expression of lipid decomposition genes, inhibits endoplasmic reticulum stress, and enhances autophagy.

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DISCLOSURES

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.

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