Nuciferine reduced fat deposition by controlling triglyceride and cholesterol concentration in broiler chickens

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ABSTRACT The purpose of this study was to investigate whether dietary nuciferine affects lipid metabolism in broiler chickens. Four treatment groups were made from 120 1-day-old broiler chickens including the base diet group (normal control [**NC**], supplemented with 0 mg/kg of nuciferine) and groups treated with 25 mg/kg, 100 mg/kg, and 400 mg/kg of dietary nuciferine, which was supplemented for 42 d. The results showed that body weight, average daily weight gain, and absolute and relative fat and liver weight were significantly decreased with nuciferine supplementation. The plasma concentration of triiodothyronine, free triiodothyronine, thyroxine, and free thyroxine was significantly decreased in the nuciferine-supplemented group, but the plasma glucagon concentration was significantly increased. The plasma and hepatic triglyceride (**TG**) and total cholesterol (**TC**) concentrations were significantly decreased in the nuciferine group, but plasma and hepatic nonesterified fatty acid concentration, hepatic lipase activity, and hepatic glycogen content were significantly increased. Hepatic histological examination showed that fat cell volume and size in the 100 and 400 mg/kg group were smaller than those in the NC group. The fatty degeneration in the liver was decreased with nuciferine supplementation. The fat cell volume and size were shrunk in the nuciferine group. Dietary nuciferine supplementation significantly decreased the gene expression level of *HMGCR*, *SREBP2*, *ACC*, and *SPEBP-1C*, but significantly increased the gene expression level of *LXR-α*, *CYP7A1*, and *CPT-I*. The results indicated that nuciferine exhibited strong reduced fat deposition activities and reflected not only by decrease of the concentration of TG and TC but also by reduction in the key gene expression level of *HMGCR*, *SREBP2*, *ACC*, and *SPEBP-1c* and elevation of the key gene expression level of *LXR-α*, *CYP7A1*, and *CPT-I*. Taken together, our results suggested that the ability of nuciferine on reducing fat deposition in broiler chickens by regulating lipid metabolism was associated with the balance of TG and TC concentration.

Key words: nuciferine, broiler chicken, liver, fat deposition

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INTRODUCTION

It is well known that if chickens have too much abdominal fat, it induces metabolic diseases and leads to adverse health effects for the consumer. As in the previous study, the reduction of excessive fatty tissue was an important factor to reduce the economic losses of many poultry producers (Wu et al., 2006). With poultry, the liver is a main organ involved in *de novo* fatty acid synthesis. In young chicks, 95% of lipids were deposited in adipose tissue or other organs (Leveille, 1969; O'Hea and Leveille, 1969). Previous studies have shown that fat deposition can be altered by regulating the gene expression involved in liver fatty acid metabolism (Stoeckman and Towle, 2002; Assaf et al., 2004), including fatty acid synthase, sterol regulatory element-binding protein (**SREBP**) 1c, acetyl-CoA carboxylase (**ACC**), ATP citrate lyase, peroxisome proliferator-activated receptor α (**PPARα**), AMP-activated protein kinase, and so on. Especially, effectively regulating gene expression in the liver that is involved in

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fatty acid metabolism can improve slaughter performance in poultry (Xie et al., 2017; Xie et al., 2019).

Worldwide, the poultry industry is one of the main agricultural subsectors (Alagawany et al., 2019). Supplementation of poultry diets with plant extracts containing bioactive components showed promising reports as feed supplements (Alagawany et al., 2018; Reda et al., 2020). Nuciferine is an aromatic ring containing alkaloid extracted from the lotus leaf (Ma et al., 2010), which is an important traditional medicine, and previous studies reported that it can exert a protective effect against liver steatosis and inflammation *in vivo* (Guo et al., 2013). Nuciferine could alleviate fructose-induced inflammation by inhibiting TLR4/PI3K/NF-kappa B signaling, which may contribute to improvement of renal injury (Wang et al., 2016). Nuciferine could inhibit Per-Arnt-Sim kinase function, which is a nutrient-responsive protein kinase, leading to the alteration in gene expression including SREBP-1c, fatty acid synthase, ACC, and lipid-responsive nuclear hormone receptor (PPARα and PPARγ) (Zhang et al., 2015). In addition, use of the plant extract to regulate the lipid profiles in the rat model and cell model was shown in previous studies of Rukkumani et al. (2005); Mahfouz et al. (2009); Pettan-Brewer et al. (2011); Metzler et al. (2013). Earlier findings stated that protein kinase B (Akt) expression was increased in oleic acid–treated cells incubated with nuciferine (Guo et al., 2013; Zhang et al., 2015) and that nuciferine improved the lipid profile and attenuated hepatic steatosis by activating the PPARα/peroxisome proliferator-activated receptor-γ coactivator pathway (Zhang et al., 2018). The other findings indicated that nuciferine attenuated hepatic steatosis by regulating lipogenesis expression, suggesting that nuciferine may play an important role during the process of triglyceride (**TG**) accumulation and was effective in decreasing fatty acid content (Zhang et al., 2015). However, the underlying mechanisms of the effects of how nuciferine regulated fat accumulation are not fully understood in broiler production. Further research is necessary to elucidate the regulatory mechanisms of nuciferine during the process of fat accumulation. Therefore, the aim of the present study was to explore the effect of nuciferine on hepatic fat metabolism, which might help to identify a possible mechanism of nuciferine in controlling TG and total cholesterol (**TC**) concentration in broiler chickens.

MATERIALS AND METHODS

Animal and Experimental Model

Male, 1-day-old broiler chickens (Ross 308, N = 120) were weighed and allocated to 4 treatment groups supplemented with 0 mg/kg (NC), 25 mg/kg, 100 mg/kg, or 400 mg/kg of dietary nuciferine. Each group includes 30 chickens. The chickens were fed with the same basal diet *ad libitum* for 42 d (the days of phase I from 1 to 21 d and the days of phase II from 22 to 42 d). The dietary nutrient levels were based on National Research Council recommended nutrient requirements for broiler chickens (Table 1). The chickens were housed in lighted coops. The temperature and humidity conditions were as per our previous study (Xie et al., 2017). All animal procedures were approved by the Fujian Agriculture and Forestry University Animal Care and Use Committee. The chickens were weighed every week to determine the average daily gain (**ADG**). Daily feed consumption was recorded per replicate to analyze the average daily feed intake and the feed conversion ratio. The chickens were deprived of feed for 12 h and weighed, and 12 chickens from each group were sacrificed at the end of the experiment. The blood was collected into heparin anticoagulant tubes, and the plasma was isolated and stored at −20°C. Subsequently, the abdominal fat pad and liver were isolated and stored at −80°C until analysis.

Measurement of the Lipid Profile

The plasma concentration of TG, TC, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol was determined using biochemical analysis methods. The concentrations of nonesterified fatty acid (**NEFA**), insulin, glucagon, triiodothyronine (**T3**), thyroxine (**T4**), free triiodothyronine, and free thyroxine (**FT4**) were measured using a commercial kit, which was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), Shanghai Langdun Bioengineering Institute (Shanghai, China), and Xiamen Suzhi Bioengineering Institute (Xiamen, China). The hepatic lipid profile was isolated from homogenized liver samples using a mixture of chloroform and methanol (2:1, v/v). The concentration of hepatic TG, TC, NEFA and glycogen and hepatic lipase (**HL**) activity were determined by using commercial kits at an

Table 1. The composition and nutrient levels of the experimental diets.

Item	Phase I	Phase II
Ingredient (%)		
Corn	56.7	61.4
Soybean meal	31.6	25.8
Corn gluten meal	3.9	4.6
Rapeseed oil	3.1	3.7
Dicalcium phosphate	1.8	1.7
Limestone	1.3	1.2
DL-Methionine	0.15	0.1
L-lysine	0.15	0.2
Premix[1]	1.0	1.0
Salt	0.3	0.3
Nutrient composition, calculated		
Metabolizable energy (kcal/kg)	3,029	3,110
Crude protein (%)	22.01	20.01
Lysine (%)	1.16	1.07
Arginine (%)	1.35	1.21
Methionine + cystine (%)	0.87	0.78
Calcium (%)	1.01	0.92
Total phosphorus (%)	0.57	0.48
Available phosphorus (%)	0.45	0.40

The nutrient level of the diets was based on National Research Council recommendations.

[1]Premix supplied the following per kilogram of diet: vitamin D3, 200 IU; vitamin A, 1500 IU; cobalamin, 0.01 mg; biotin, 0.15 mg; vitamin K3, 0.5 mg; folic acid, 0.55 mg; thiamine, 1.8 mg; pyridoxine, 3.5 mg; riboflavin, 3.6 mg; vitamin E, 10 mg; D-pantothenic acid, 10 mg; niacin, 35 mg; Se, 0.15 mg; I, 0.35 mg; Cu, 8 mg; Zn, 40 mg; Mn, 60 mg; Fe, 80 mg.

Table 2. The primer sequence and parameters.

Genes	NCBI ID no.	Primer sequence (5'→3')	Product length (bp)	Annealing temperature (°C)
β-actin	GenBank: L08165.1	GTGTGATGGTTGGTATGGGC CTCTCTTGGCTTTGGGGTTC	225	59
CPT-I	GenBank: AY675193.1	GAAGACGGAACACTGCAAAGG GGGCAAGTTGAATGAAGGCA	223	60
ACC	NCBI Reference Sequence: NM_205505.1	GCTGGGTTGAGCGACTAATG GGGAAACTGGCAAAGGACTG	173	59
SREBP-1c	GenBank: AY029224.1	TGGCTGAGATCTATGTGGCG GAAGAAGCGGTGACCCAATG	173	59
SREBP-2	GenBank: AJ414379.1	AAGCAACCAGACCCACCTAA GCCAGCTTCAGAACCATGTT	230	59
HMGCR	GenBank: AB109635.1	GCCGTATTCTTGCAGATGGG CATTCCCATTGCATCCCCTG	230	60
LXR-α	GenBank: AJ507202.1	ATAGCAAGCCCTTCCTGTGT ACGTTGTAGTGGAAGCCTGA	248	59
CYP7α1	NCBI Reference Sequence: NM_001001753.1	TGACCCAGCAGAAGGAAACA ACCCAGGTGTTAGGCTGAAA	159	59

Abbreviations: ACC, acetyl CoA carboxylase; CPT-I, carnitine palmitoyltransferase I; CYP7α1, cytochrome P450 family 7 subfamily A member 1; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; LXR-α, liver X receptor α (LXR-α); SREBP-1c, sterol regulatory element-binding protein 1c; SREBP-2, sterol regulatory element-binding protein 2.

absorbance of 550 and 440 nm, respectively. One unit of HL enzyme activity represents 1 mg of tissue protein releasing 1 μmol of fatty acid per hour.

Histopathological Analysis

The paraformaldehyde-fixed livers were stained with hematoxylin and eosin or periodic acid–Schiff. Morphological analysis was analyzed by microscopic fields per section using the computerized image analysis system.

RNA Extraction, cDNA Synthesis, and Real-Time Quantitative PCR Detecting System

Total RNA was extracted from the liver tissue samples using the TRIzol reagent (Invitrogen, Carlsbad, CA) and as per the instruction manual. The RNA concentration was quantified by measuring the absorbance at 260 nm in a spectrophotometer (Eppendorf Biotechnology, Hamburg, Germany). The mRNA expression levels were determined by real-time quantitative PCR detecting system on a MyiQ2 PCR system (Bio-Rad, Hercules, CA) and analyzed using the $2^{-\triangle\triangle Ct}$ method. The primers were designed using Primer Premier 5.0 software (Shanghai, China) and synthesized by Sangon Biotech (Shanghai, China), and they are listed in Table 2.

Statistical Analysis

One-way analysis of variance (Statistical Packages for Social Science 20.0; IBM, Corporation, Armonk, NY) was used to analyze the data via a multiple range test. A replicate was considered the experimental unit for the entire index determined. The results were expressed as mean ± SEM. $P < 0.05$ or $P < 0.01$ shows the significant or extremely significant differences.

RESULTS

Growth Performance and Carcass Composition

As shown in Table 3, compared with the control group, the chickens fed with 400 mg/kg of nuciferine showed a significantly lower body weight (**BW**) and ADG ($P < 0.05$), but the other group only showed a downward trend. However, the average daily feed intake and feed conversion ratio were not affected during the whole study period in groups treated with nuciferine ($P > 0.05$). The absolute and relative abdominal fat weights were decreased in the groups treated with a dose of 100 and 400 mg/kg of nuciferine compared with the control group ($P < 0.05$). In addition, the absolute abdominal fat weight was significantly decreased when compared with the group supplemented with 25 mg/kg of nuciferine ($P < 0.05$). The absolute and relative liver weights were significantly lower in the group treated with 400 mg/kg of nuciferine than in the control group and the group treated with 25 mg/kg of nuciferine ($P < 0.05$).

Plasma Hormone Concentration

As shown in Table 4, the plasma concentration of T3, T4, and FT4 was significantly decreased in the group treated with 100 and 400 mg/kg of nuciferine compared with the control group ($P < 0.05$ or $P < 0.01$, respectively). The plasma concentration of free triiodothyronine was significantly decreased ($P < 0.05$) in the groups supplemented with 3 doses of nuciferine. There was no difference in terms of plasma insulin content among the groups supplemented with nuciferine ($P > 0.05$). The plasma glucagon concentration was significantly increased in the group treated with 400 mg/kg of nuciferine ($P < 0.05$).

Table 3. Effect of nuciferine on growth performance and carcass composition in broiler chickens.

Item	Supplemental nuciferine (mg/kg)			
	0 mg/kg	25 mg/kg	100 mg/kg	400 mg/kg
BW (g), 42 d	2,631.39 ± 16.18	2,618.4 ± 20.03	2,537.46 ± 33.75	2,428.68 ± 31.48*
ADG (g/d), 22–42 d	62.65 ± 4.13	62.34 ± 12.04	60.42 ± 7.15	56.83 ± 3.01*
ADFI (g/d), 22–42 d	145.5 ± 5.03	142.68 ± 8.12	141.22 ± 4.35	139.32 ± 6.11
FCR (g:g), 22–42 d	1.98 ± 0.05	1.94 ± 0.11	1.92 ± 0.04	1.96 ± 0.07
Absolute abdominal fat (g)	124.8 ± 10.8	116.5 ± 9.6	98.7 ± 7.4*	93.4 ± 14.8*,**
Relative abdominal fat (%)	4.74 ± 0.67	4.45 ± 0.48	3.89 ± 0.22*	3.85 ± 0.47*
Absolute liver weight (g)	59.9 ± 5.8	54.6 ± 7.1	51.9 ± 9.4	46.3 ± 2.5*,**
Relative liver weight (%)	2.28 ± 0.30	2.20 ± 0.35	2.05 ± 0.34	1.91 ± 0.08*,**

Data are presented as means ± SEM. Each nuciferine-treated group represents 12 chickens at the age of 42 d.

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; BW, body weight; FCR, feed conversion ratio.

*$P < 0.05$ indicates statistically significant differences when compared with the control group.

**$P < 0.05$ indicates statistically significant differences when compared with the group treated with 25 mg/kg of nuciferine.

Hepatic and Plasma Lipid Profile

As shown in Table 5, the plasma and hepatic TG concentrations were significantly decreased in the groups treated with 100 and 400 mg/kg of nuciferine ($P < 0.05$ and $P < 0.01$, respectively) compared with the control group; in addition, a difference ($P < 0.05$ and $P < 0.01$, respectively) was shown between the groups supplemented with 100 and 400 mg/kg and the group supplemented with 25 mg/kg of nuciferine. The plasma TC concentration was obviously decreased ($P < 0.05$) between the group treated with 400 mg/kg of nuciferine and the control group. The plasma and hepatic NEFA concentration was significantly decreased in the groups treated with 100 and 400 mg/kg of nuciferine compared with the control group; meanwhile, the hepatic NEFA concentration was also decreased when compared with the group treated with 25 mg/kg of nuciferine ($P < 0.05$ or $P < 0.01$, respectively). The plasma low-density lipoprotein content was significantly lower in the groups treated with 100 and 400 mg/kg of nuciferine ($P < 0.05$ or $P < 0.01$, respectively), but no difference was observed in terms of plasma high-density lipoprotein content among the entire nuciferine-supplemented groups ($P > 0.05$). The HL activity was significantly increased in the groups treated with 100 and 400 mg/kg of nuciferine compared with the control group and the group treated with 25 mg/kg of nuciferine ($P < 0.01$). The hepatic glycogen content was significantly increased in the groups treated with 100 and 400 mg/kg of nuciferine ($P < 0.05$) compared with the control group.

Histological Analysis

Hepatic histological examination showed a central vein located in the center of the hepatic lobe, a central vein was radiating and arranged by the hepatic cord or plate. Interval the hepatic cords or masses are irregular and the variable sinusoid was shown in the liver. Importantly, the liver cell showed severe fatty degeneration. Cytoplasm showed abundant and large lipid droplets within hepatocytes and lipid vacuoles. By contrast, the fat cell volume and size in the group treated with 100 and 400 mg/kg of nuciferine were smaller than in the NC group. Importantly, the degree of hepatocyte degeneration is the lowest in the group treated with 400 mg/kg of nuciferine (Figure 1).

Lipid Metabolism

As shown in Figure 2, the results showed that the expression of 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*), *SREBP2*, *ACC*, and *SPEBP-1c* was significantly decreased in the group treated with 400 mg/kg of nuciferine ($P < 0.05$ and $P < 0.01$,

Table 4. Effect of nuciferine on plasma metabolic hormones in broiler chickens.

Item	Supplemental nuciferine (mg/kg)			
	0 mg/kg	25 mg/kg	100 mg/kg	400 mg/kg
T3 (ng/mL)	1.68 ± 0.15	1.71 ± 0.11	1.59 ± 0.16*	1.49 ± 0.14*
T4 (ng/mL)	49.32 ± 3.01	47.01 ± 1.89	44.32 ± 1.05*	37.89 ± 1.94*
FT3 (fmol/mL)	3.09 ± 0.32	2.31 ± 0.12*	2.11 ± 0.29*	1.99 ± 0.35**
FT4 (fmol/mL)	8.11 ± 0.49	7.81 ± 0.51	6.38 ± 0.47*	6.21 ± 0.78*
Insulin (pg/mL)	207.45 ± 11.23	209.21 ± 18.76	211.63 ± 20.07	210.45 ± 29.11
Glucagon (pg/mL)	278.91 ± 9.71	284.44 ± 16.73	296.52 ± 14.42	311.04 ± 9.65*

Data are presented as means ± SEM. Each nuciferine-treated group represents 12 chickens at the age of 42 d.

Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; T3, triiodothyronine; T4, thyroxine.

*$P < 0.05$ indicates statistically significant differences when compared with the control group.

**$P < 0.01$ indicates statistically significant differences when compared with the control group.

Table 5. Effect of nuciferine on the plasma and hepatic lipid profile in broiler chickens.

Item	Supplemental nuciferine (mg/kg)			
	0 mg/kg	25 mg/kg	100 mg/kg	400 mg/kg
Plasma TG (mmol/L)	0.68 ± 0.09	0.64 ± 0.11	0.59 ± 0.06*,#	0.45 ± 0.04**,##
Hepatic TG (mmol/g)	2.32 ± 0.41	2.11 ± 0.81	1.94 ± 0.20*	1.89 ± 0.19*
Plasma TC (mmol/L)	3.68 ± 0.37	3.57 ± 0.51	3.35 ± 0.36	3.22 ± 0.21*
Plasma NEFA (μmol/L)	899.38 ± 23.35	847.01 ± 61.02	844.2 ± 101.5*	837.91 ± 91.04*
Hepatic NEFA (μmol/L)	53.09 ± 4.32	52.31 ± 9.08	40.15 ± 5.89*,#	36.04 ± 7.53**,##
Plasma LDL (mmol/L)	0.96 ± 0.13	0.91 ± 0.07	0.81 ± 0.06*	0.71 ± 0.04**
Plasma HDL (mmol/L)	1.52 ± 0.23	1.47 ± 0.28	1.42 ± 0.35	1.37 ± 0.25
HL (U/mg)	2.07 ± 0.23	2.09 ± 0.46	3.79 ± 0.17**,##	3.45 ± 0.11**,##
Hepatic glycogen (mg/g)	2.91 ± 0.71	3.14 ± 0.73	4.07 ± 0.39*	3.94 ± 0.45*

Data are presented as means ± SEM. Each nuciferine-treated group represents 12 chickens at the age of 42 d.

Abbreviations: HDL, high-density lipoprotein; HL, hepatic lipase; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; TC, total cholesterol; TG, triglycerides.

*$P < 0.05$ indicates statistically significant differences when compared with the control group.

**$P < 0.01$ indicates statistically significant differences when compared with the control group.

#$P < 0.05$ indicates statistically significant differences when compared with the group treated with 25 mg/kg of nuciferine.

##$P < 0.01$ indicates statistically significant differences when compared with the group treated with 25 mg/kg of nuciferine.

respectively), but *SREBP2* and *SREBP-1c* expression was also significantly ($P < 0.05$) downregulated in the group treated with 100 mg/kg of nuciferine compared with those in the control group. The *LXR-α*, cytochrome P450 family 7 subfamily A member 1 (*CYP7A1*), and *CPT-I* expression was significantly increased ($P < 0.05$ and $P < 0.01$, respectively) in the groups treated with 100 and 400 mg/kg of nuciferine. Importantly, the group supplemented with 400 mg/kg of nuciferine also showed significant increase in the expression levels of *LXR-α*, *CYP7A1*, and *CPT-I* compared with the group supplemented with 25 mg/kg of nuciferine ($P < 0.05$ and $P < 0.01$, respectively).

DISCUSSION

In the present study, the BW and ADG of broiler chickens were significantly reduced in the nuciferine-supplemented groups compared with the control group. Importantly, nuciferine supplementation could reduce the absolute and relative abdominal fat and liver weight. These results were similar to those of a previous study

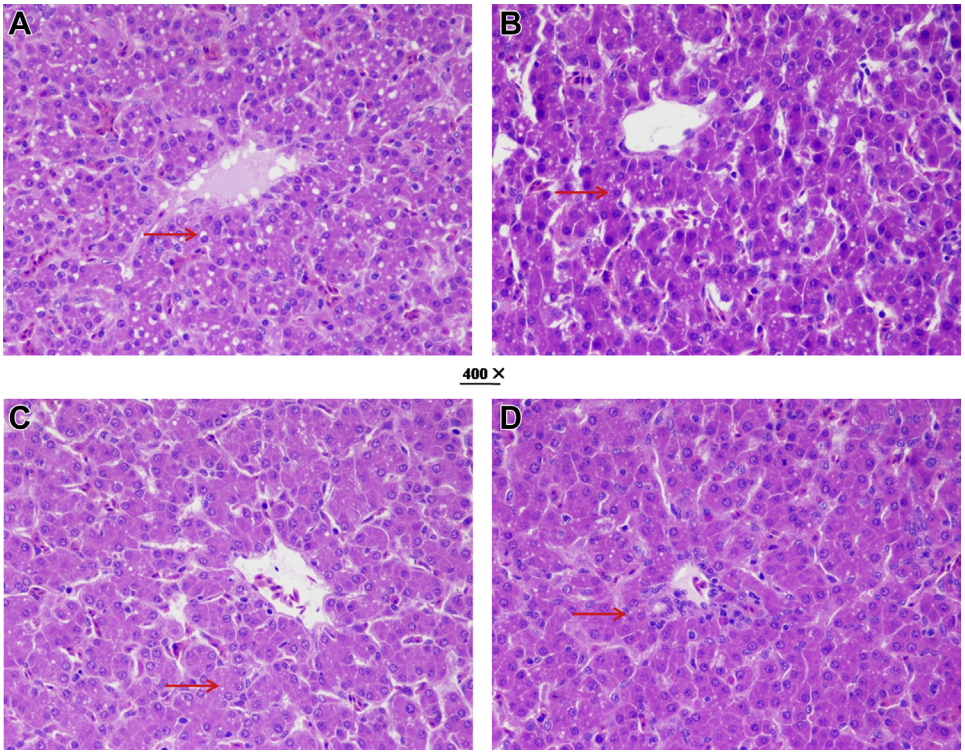

Figure 1. The effect of nuciferine on hepatic histology in broiler chickens: (A) control group; (B) group supplemented with 25 mg/kg of nuciferine; (C) group supplemented with 100 mg/kg of nuciferine; (D) group supplemented with 400 mg/kg of nuciferine.

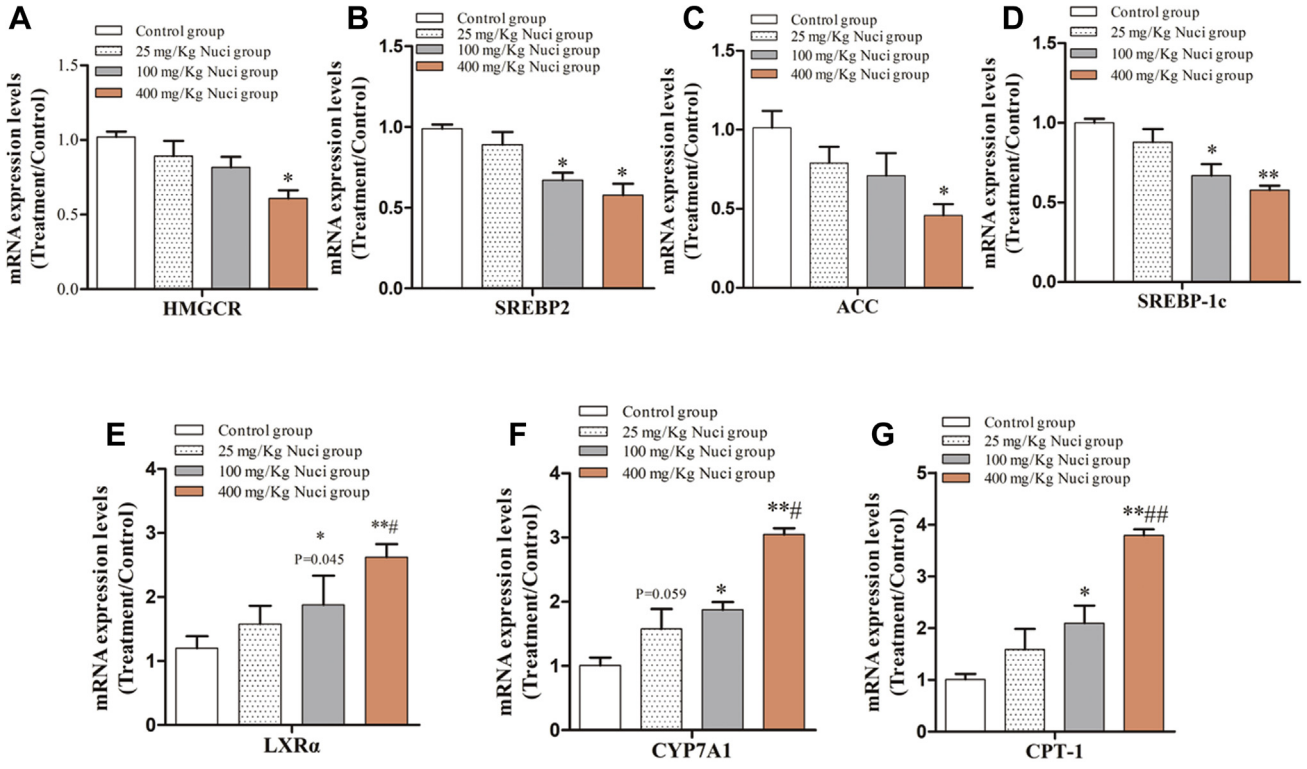

Figure 2. The effect of nuciferine on gene expression including key gene expression levels of (A) *HMGCR*, (B) *SREBP2*, (C) *ACC*, (D) *SPEBP-1c*, (E) *LXR-α*, (F) *CYP7A1*, and (G) *CPT-I* in the liver of broiler chickens. The experiments used 12 samples from each group. Data are expressed as mean ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences from the control group. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ indicate significant differences from the group supplemented with 25 mg/kg of nuciferine. Abbreviations: ACC, acetyl CoA carboxylase; CPT-I, carnitine palmitoyltransferase I; CYP7α1, cytochrome P450 family 7 subfamily A member 1; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; LXR-α, liver X receptor α; SREBP2, sterol regulatory element-binding protein 2; SREBP-1c, sterol regulatory element-binding protein 1c.

associated with plant extract function, in which male Zucker obese rats treated with high doses of *Garcinia cambogia* extract could effectively suppress fat accumulation (Saito et al., 2005). Previous studies also reported that curcumin safely promotes weight loss in laboratory animals (Kang et al., 2011) and humans (Di Pierro et al., 2015) and suggest that the regulation of food intake may be a major weight management mechanism of curcumin. Therefore, it appears that broiler chickens would likely benefit from nuciferine owing to the suppression of body fat accumulation.

In the present study, the plasma concentration of T3, T4, and FT4 was significantly decreased in the groups treated with 100 and 400 mg/kg of nuciferine. Triiodothyronine, T4, and FT4 as constituents of thyroid hormones are important and key metabolic hormones associated with energy production (Hornick et al., 2000; Smith et al., 2002). Previous studies indicated that reducing circulating thyroid hormone levels is a protective mechanism for the body's energy reserves (Buyse et al., 2002). Presumably, the nuciferine treatment of broiler chickens decreased thermogenesis, thus resulting in decrease in the body's energy reserves. Thus, nuciferine affected BW through regulation of the plasma content of thyroid hormones in broiler chickens, thus controlling fat accumulation.

In the present study, the weights of abdominal and relative liver weight were significantly decreased in the group treated with 400 mg/kg of nuciferine. Our result was similar to a previous result which also shown the relative liver weight reduction in rats (Kim et al., 2008). This change may be control fat accumulation, as shown the results of hepatic histological. Hepatic lipogenesis resulted from the increased influx of free fatty acids into hepatocytes, which is a crucial risk factor leading to deterioration of fatty liver. However, nuciferine alleviated the accumulation of lipid droplets within hepatocytes, and the hepatocytes showed severe fatty degeneration. Guo et al. (2013) measured the concentration of TG and free fatty acid of hepatic tissue extracted from high-fat diet–fed hamsters and found that nuciferine had a function in downregulation of fat accumulation. Similarly, as shown in our result, nuciferine supplementation could independently and significantly reduce the plasma and hepatic concentration of TG and TC, leading to decrease or inhibition of fat accumulation.

Fat accumulation is a complex process associated with the metabolism of TG and TC. The CoA carboxylase is a key enzyme of fatty acid synthesis and fatty acid production, which could transport the acetyl-CoA from the extramitochondrial cleavage of citrate (Brownsey et al., 2006). The level of *ACC* mRNA expression was significantly decreased with nuciferine supplementation. In addition, mRNA expression of *SREBP-1c* was reduced after the administration of nuciferine. Previous studies have shown

that SREBP is an important nuclear transcript factor in lipid metabolism, which possess the following 3 subtypes: SREBP-1a, SREBP-1c, and SREBP-2 (Brown and Goldstein, 1997; Stoeckman and Towle, 2002; Kong et al., 2004; Bursill and Roach, 2006). Sterol regulatory element-binding protein 1c mainly plays an important role in regulating lipogenic enzyme gene expression (Stoeckman and Towle, 2002; Assaf et al., 2004), but SREBP-2 is mainly involved in cholesterol metabolism (Brown and Goldstein, 1997). The other important protein of HMGCR is a rate-limiting enzyme in the process of cholesterol synthesis in hepatocytes, which catalyzes the formation of methyl valerate (Campia et al., 2009). Recent research also indicated that HMGCR protein function was inhibited or degradation can reduce the accumulation of cholesterol (Jiang et al., 2018). In the present study, nuciferine supplementation significantly decreased the gene expression levels of *HMGCR*, *SREBP2*, *ACC*, and *SPEBP-1c*. Therefore, the alteration in these gene expressions might be directly responsible for the effect of nuciferine on reducing the TC and TG synthesis in broiler chickens.

Previous studies had presumed that increased fatty acid oxidation in the liver of an animal is an important step for inhibition of fat accumulation (Xie et al., 2017; Xie et al., 2019). Using nutritional antioxidants in livestock systems is considered the key in improving animal production (Elwan et al., 2019). Thus, we presume that the mechanism of nuciferine reducing fat deposition could be due to the increase in TG and TC oxidation in broiler chickens. The present study showed that nuciferine treatment caused a significant upregulation of liver X receptor α (**LXR-α**), CYP7A1, and carnitine palmitoyltransferase I (**CPT-I**) expression in broiler liver tissue. Liver X receptor α and CYP7A1 are important proteins during the process of limiting the transcription level and protein processing of SREBP (Yoshikawa et al., 2001). Tobin et al. (2002) also reported that the LXR gene in mice when knocked out significantly inhibited expression of fatty acid and cholesterol metabolism enzymes and limited fat accumulation. In addition, previous results indicated that activating CPT-I could increase fat oxidation via inhibition of malonyl CoA formation (Ishihara et al., 2000). Considering the decreased TG and TC content in plasma and the liver after nuciferine treatment, it is possible that nuciferine improvement in gene expression of *LXR-α*, *CYP7A1*, and *CPT-I* in the liver led to inhibition of the process of fat formation and increase in the oxidation of fatty acids.

CONCLUSIONS

In the present study, we systematically evaluated the reduced fat deposition activities of nuciferine in vivo. The results indicated that nuciferine exhibited strong reduced fat deposition activities. Its reduced fat deposition activities was reflected not only by decrease in the concentration of plasma TC and TG but also in reduction in the release levels of cholesterol and lipid anabolism key genes HMGCR, SREBP2, ACC, and SPEBP-1c and elevation in release levels of cholesterol and lipid catabolism key genes LXR-α, CYP7A1, and CPT-I. Taken together, our results suggested the ability of nuciferine in reducing fat deposition in broiler chickens.

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