Genistein activated adenosine 5′-monophosphate-activated protein kinase–sirtuin1/peroxisome proliferator-activated receptor γ coactivator-1α pathway potentially through adiponectin and estrogen receptor β signaling to suppress fat deposition in broiler chickens

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ABSTRACT Genistein can be used as a dietary additive to control fat deposition in animals, while its mechanism is poorly understood. In this study, a total of 144 male broilers were randomly divided into 4 groups. Birds were fed standard diets supplemented with 0, 50, 100 or 150 mg of genistein/kg from 21 to 42 d of age. Results showed that genistein treatment decreased the relative weight of abdominal fat and triglyceride contents in broiler chickens. Genistein downregulated hepatic lipid droplets accumulation and upregulated the activity of lipoprotein lipase and hepatic lipase and the concentration of adiponectin. Furthermore, the liver X receptor α, sterol regulatory element–binding protein 1c (**SREBP-1c**), acetyl-CoA carboxylase (**ACC**), and fatty acid synthase (**FAS**) mRNA expressions were decreased, whereas adiponectin receptor 2, peroxisome proliferator-activated receptor α, adipose triglyceride lipase, and carnitine palmitoyl transferase-I (**CPT-I**) mRNA abundances were increased in the liver of broilers treated with genistein. In addition, genistein increased the NAD^+ concentration and NAD^+/NADH ratio in the liver. Genistein increased estrogen receptor β (**ERβ**), forkhead box O1, nicotinamide phosphoribosyl transferase, sirtuin1 (**SIRT1**), phospho (**p**)-adenosine 5′-monophosphate-activated protein kinase (**AMPK**), peroxisome proliferator-activated receptor γ coactivator-1α (**PGC-1α**), p-ACC, and CPT-I protein levels, whereas the SREBP-1c and FAS levels were decreased. These data indicated that genistein might reduce fat accumulation in broiler chickens *via* activating the AMPK-SIRT1/PGC-1α signaling pathway. The activation of this signaling pathway might be achieved by its direct effect on improving the adiponectin secretion or its indirect effect on upregulation of ERβ expression level through paracrine acting of adiponectin.

Key words: AMPK-SIRT1/PGC-1α, broiler chicken, genistein, lipid metabolism

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INTRODUCTION

The chicken is beneficial to people's health because of its nutritional advantages of high protein and low fat. In recent decades, the growth rate and feed conversion efficiency of chickens have been greatly improved to satisfy the demand of consumers. However, commercial chickens exhibiting excessive fat accumulation has become a major problem in the modern poultry industry and harms the economic interests of the poultry industry (Baéza and Le, 2013). More importantly, the consumption of high-fat meat products will increase the risk of obesity and other metabolic diseases (Balaji et al., 2016). Owing to the adverse effects of excess fat deposition in chickens, numerous research studies have been implemented to solve the issue. Diets supplemented with plant extract may be an effective way to improve the quality of chicken (Han et al., 2016; Farahat et al., 2017).

In addition to the nutritional value, researchers have proved that soybean can regulate lipid metabolism because it contains a high concentration of isoflavone (Kwon et al., 2010). Genistein is the most abundant of isoflavone in soybean and is also regarded as one kind of polyphenols (Palacios-González et al., 2014).

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Lv et al. (2018) reported that dietary supplemented with genistein could downregulate lipid synthesis–related gene expression in chickens, such as sterol regulatory element-binding protein 1c (**SREBP-1c**) and fatty acid synthase (**FAS**). Genistein could also improve fatty acid β-oxidation in obesity rats by enhancing peroxisome proliferator-activated receptor α (**PPARα**), adipose triglyceride lipase (**ATGL**) and carnitine palmitoyl transferase-I (**CPT-I**) expression levels (Huang et al., 2016). Although studies have certified that genistein poses beneficial effects on fat reduction in animals, the precise physiological mechanism, especially its action on broiler chickens, is still ambiguous.

The liver is the primary organ responsible for lipid and energy metabolism. Adenosine 5′-monophosphate-activated protein kinase (**AMPK**) and sirtuin1 (**SIRT1**) play an essential role in liver lipid metabolism (Hardie et al., 2012; Simmons et al., 2015). Studies had reported that the activation of AMPK and SIRT1 could regulate lipogenesis-related transcription factors, which affect the activity of lipid metabolism enzyme (e.g., FAS and ACC) expression (Chen et al., 2013). In addition, they could also improve the fatty acid β-oxidation through enhancing peroxisome proliferator-activated receptor γ coactivator-1α (**PGC-1α**) expression level (Cantó et al., 2009; Higashida et al., 2013). Thus, in this study, we investigated the effect of genistein on lipid metabolism–related factors in broiler chickens and further evaluated whether this action of genistein is associated with the activation of the AMPK-SIRT1/PGC-1α signaling pathway.

However, the mechanism of genistein to activate AMPK and SIRT1 is still unknown in broiler chickens. Research studies have proved that AMPK could affect the expression of SIRT1 by regulating the NAD^+ metabolism, whereas SIRT1 could also enhance the phosphorylation of AMPK (Cantó et al., 2010; Palacios-González et al., 2014; Yao et al., 2018). In addition, previous studies have reported that estrogen and adiponectin could regulate the expression of AMPK and SIRT1 (You and Rogers, 2009; Guo et al., 2017). Genistein has estrogen-like effects and could improve adiponectin secretion (Behloul and Wu, 2013; Ganai and Farooqi, 2015), which might be its way to activate AMPK and SIRT1 in broiler chickens. Therefore, in this study, we also analyzed the content of adiponectin in serum and the expression of estrogen receptors in the liver to reveal the possible mechanism of genistein to activate the AMPK-SIRT1 signaling pathway. These results will contribute to the revelation of the specific mechanism of genistein on lipid metabolism in broiler chickens but also remains to be further verified at the cellular level.

MATERIALS AND METHODS

Experimental Design and Diets

This study was approved by the Animal Ethics Committee of the University of Nanjing Agricultural (IACUC2018014). A total of 144 male Ross 308 broiler chickens at 1 d of age were purchased from the Jinghai Poultry Company (Nantong, China) and were caged under a 12L:12D cycle, in a temperature- and humidity-controlled environment. The temperature was maintained at 34°C for the first 5 d and then gradually decreased by 3 to 5°C every 7 d until it reached 22°C. The RH was maintained at 60 to 70% in the first 10 d and at 50 to 60% in the later period of the experiment. From 1 to 20 d of age, all broiler chickens were fed on the same starter diet (Table 1). Then, broiler chickens were randomly divided into 4 groups (12 replicates of 3 birds each) based on their BW. From 21 to 42 d of age, 4 groups of chickens were fed finisher diets containing genistein at 0 mg/kg, 50 mg/kg, 100 mg/kg, or 150 mg/kg diets. Genistein (extracted from *Glycine max*) was purchased from Sigma (St Louis, MO): purity (HPLC), 995 mg/g; water, 4 mg/g; elemental anal, 667.9 mg/g. Premixes of the 4 doses of genistein were prepared and added to the standard diet and mixed homogenously in a feed mixer to obtain the diets. BW was weighed at 21 and 42 d of age to determine ADG. Feed remaining was weight to calculate ADFI and feed conversion ratio. At the end of the experiment, chickens were randomly selected, bled via the brachial vein for blood samples, and then slaughtered and dissected by a trained team. The abdominal fat, liver, breast and leg muscle were subsequently removed and weighed. Blood samples were centrifuged at 3,000 × *g* for 10 min before collecting the serum. All samples were stored at −80°C for further analysis.

Determination of Lipid Parameters

The contents of triglyceride (**TG**), free fatty acid (**FFA**), glycerin, and low-density lipoprotein cholesterol and the activity of lipoprotein lipase in serum were measured following the manufacturer's protocol. In addition, the content of TG, FFA, glycerin, and hepatic lipase activity in the liver were measured using commercial kits as per the manufacturer's instructions. All commercial kits were obtained from Nanjing Jiancheng Biotechnology Institution (Nanjing, China).

Assessment of Lipid Accumulation by Oil Red O Staining

Information regarding the assessment of lipid accumulation by Oil red O staining is shown in Supplementary Materials.

Determination of Adiponectin Concentration

Serum adiponectin concentration was detected using ELISA kits following the manufacturer's protocol. The chicken adiponectin content detection ELISA kit was obtained from Feiya Biotechnology (Yangzhou, China).

Table 1. Ingredient and nutrient composition of the diets for the broiler chickens.

Item	Starter (1–20 d)	Finisher (21–42 d)
Ingredient (g/kg)		
Corn	526	574
Wheat bran	20	40
Soybean meal	311	270
Rapeseed oil	50	50
Fish meal	60	30
Salt	3	3
Calcium phosphate	10	15
Limestone	12	12
DL-Methionine	3	1
Premix[1]	5	5
Calculated nutrient levels[2] (g/kg)		
ME (kcal/kg)	3,100	3,140
DM	892.7	895.6
CP	225.2	197.4
Lysine	11.9	10.8
Methionine + cysteine	9.3	7.1
Calcium	10.0	9.0
Total phosphorus	8.0	7.6
Available phosphorus	4.7	3.9

The nutritional level and needs of the broiler chickens' diets were based on NRC recommendations (NRC, 1994).

[1]Premix supplied the following per kilogram of diet: vitamin A, 1,500 IU; vitamin D3, 200 IU; vitamin E, 10 mg; vitamin K3, 0.5 mg; thiamine, 1.8 mg; riboflavin, 3.6 mg; D-pantothenic acid, 10 mg; folic acid, 0.55 mg; pyridoxine, 3.5 mg; niacin, 35 mg; cobalamin, 0.01 mg; biotin, 0.15 mg; Fe, 80 mg; Cu, 8 mg; Mn, 60 mg; Zn, 40 mg; I, 0.35 mg; Se, 0.15 mg.

[2]The nutrient values were calculated based on the analyzed nutrient values as per the NRC (1994).

Measurement of NAD^+ Content and NAD^+/NADH Ratio

Liver tissues were homogenized on ice with either NAD^+ or NADH extraction buffer and centrifuged at 2,500 × *g* for 10 min at 4°C to collect the supernatants. The concentration of NAD^+ or NADH in supernatants was detected using a commercial kit following the manufacturer's protocol. Then, the NAD^+/NADH ratio was analyzed. These commercial kits were obtained from Abbkine (Wuhan, China).

Determination of Lipid Metabolism–Related Gene Expression Level

Information regarding the determination of lipid metabolism–related gene expression level is shown in Supplementary Materials.

Western Blotting Analysis

Information regarding the determination of Western blotting analysis is shown in Supplementary Materials.

Statistics Analysis

Data are expressed as mean ± SEM. Differences were evaluated by 1-way ANOVA and independent-sample *t* test using SPSS statistics software (version 20.0 for Windows; SPSS Inc., Chicago, IL), and the differences were considered significant at $P < 0.05$.

RESULTS

Effect of Genistein on Growth Performance

As shown in Table 2, no statistical difference among BW, ADFI, and feed conversion ratio was observed in genistein treatment groups compared with the control group ($P > 0.05$). In addition, no significant difference was observed in the relative weight of the breast, leg muscle, and liver either between 4 groups ($P > 0.05$) (Table 2). However, supplementation with 100 and 150 mg/kg genistein significantly decreased the relative weight of abdominal fat compared with the control group ($P < 0.05$) (Table 2).

Effect of Genistein on Serum Lipid Metabolism–Related Parameters

Compared with the control group, supplementation with 100 and 150 mg/kg genistein significantly decreased TG and low-density lipoprotein cholesterol content in serum ($P < 0.05$) (Table 2). In contrast, the contents of FFA and glycerol in serum were significantly increased ($P < 0.05$) (Table 2). Furthermore, lipoprotein lipase activity in serum was significantly enhanced in the 150 mg/kg genistein treatment group compared with the control group ($P < 0.05$) (Table 2).

Impact of Genistein on Lipid Droplets Accumulation in the Liver

The statistical analysis showed that supplementation with 100 and 150 mg/kg genistein significantly decreased the counts and total area of lipid droplets in the liver compared with the control group ($P < 0.01$) (Figure 1). Similarly, biochemical analysis results showed that compared with the control group, the content of TG in the liver was significantly decreased in the 100 and 150 mg/kg genistein treatment groups ($P < 0.05$) (Table 2). In contrast, the content of FFA and glycerol in the liver was significantly increased ($P < 0.05$) (Table 2). Meanwhile, hepatic lipase activity in the liver was significantly enhanced in the 150 mg/kg genistein treatment group compared with the control group ($P < 0.05$) (Table 2).

Impact of Genistein on Lipogenesis-Related Gene Expression Levels in the Liver

Supplementation with 100 and 150 mg/kg genistein decreased the *LXRα* and *SREBP-1c* mRNA levels compared with the control group ($P < 0.05$). As their downstream factors, the mRNA levels of *FAS* and *ACC* mRNA were also declined in 100 and 150 mg/kg

Table 2. Effect of genistein on growth performance and lipid parameters during 21 to 42 d of age.

Item	Supplementation with genistein (mg/kg)				SEM	*P* Value	
	0	50	100	150		Linear	Quadratic
Growth performance							
BW (g) at age of 21 d	477.08	478.94	475.76	480.32	2.044	0.729	0.750
BW(g) at age of 42 d	1,840.16	1,814.33	1,858.04	1,821.25	15.776	0.928	0.866
ADG (g/day)	64.91	63.59	65.82	63.85	0.763	0.894	0.835
ADFI (g/day)	116.86	119.9	117.9	121.1	1.294	0.311	0.634
FCR(g/g)	1.80	1.85	1.79	1.89	0.022	0.285	0.539
RWAF (g/100g of BW)	2.50	2.41	2.26*	2.20*	0.047	0.012	0.810
RWLM (g/100g of BW)	7.38	7.6	7.23	7.14	0.093	0.11	0.283
RWB (g/100g of BW)	14.00	13.90	13.06	14.03	0.182	0.703	0.156
RWL (g/100g of BW)	2.72	2.54	2.82	2.67	0.523	0.726	0.927
Lipid parameters in serum							
TG (mmol/L)	0.37	0.32	0.24*	0.27*	0.031	0.048	0.532
FFA (mmol/L)	1.04	1.10	1.25*	1.26*	0.047	0.001	0.457
Glycerol (μmol/L)	13.24	15.66	18.16*	18.03*	2.185	0.024	0.418
LDL-C (mmol/L)	3.03	2.95	2.31**	2.38*	0.105	0.003	0.657
LPL (U/mL)	5.28	5.26	5.49	6.60*	0.221	0.007	0.303
Lipid parameters in the liver							
TG (mmol/g)	1.87	1.64	1.42*	1.42*	0.064	0.003	0.332
FFA (μmol/g)	63.25	69.04	75.69*	78.68*	5.483	0.006	0.906
Glycerol (μmol/g)	70.03	82.96	98.18*	93.41*	10.468	0.018	0.236
HL (U/mg)	5.07	5.47	5.54	6.00*	0.158	0.049	0.913

Values are means ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, compared with the control group.

Abbreviations: FCR, feed conversion ratio; FFA, free fatty acid; HL, hepatic lipase; LDL-C, low-density lipoprotein cholesterol; LPL, lipoprotein lipase; RWAF, relative weight of abdominal fat; RWB, relative weight of breast; RWL, relative weight of liver; RWLM, relative weight of leg muscle; TG, triglyceride.

genistein treatment groups compared with that of the control group ($P < 0.05$) (Figure 2A). Moreover, genistein treatment enhanced the p-ACC protein level ($P < 0.05$) (Figure 2D). Conversely, the protein levels of SREBP-1c and FAS were significantly decreased in 100 and 150 mg/kg genistein treatment groups compared with that of the control group ($P < 0.05$) (Figures 2F and 2G).

Impact of Genistein on Lipolysis-Related Gene Expression Levels in the Liver

The mRNA levels of *PPARα*, *ATGL*, and *CPT-I* were increased in the 100 and 150 mg/kg genistein treatment groups compared with the control group ($P < 0.05$) (Figure 2B). Meanwhile, the CPT-I protein level was increased in the 100 and 150 mg/kg genistein treatment

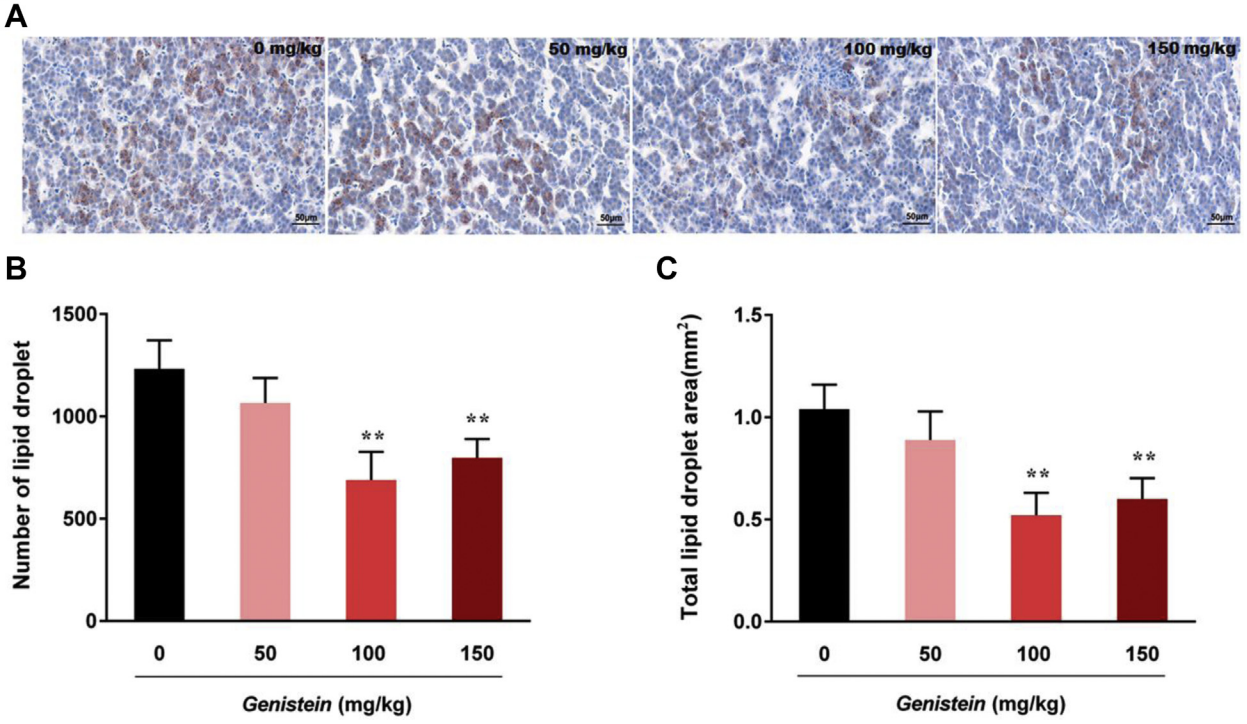

Figure 1. Effects of genistein on lipid droplet accumulation in the liver. (A) Representative photomicrographs of Oil Red O staining; (B) Quantitation of lipid droplets; (C) Total area of lipid droplets. Data are expressed as means ± SEM. $^{**}P < 0.01$, compared with the control group.

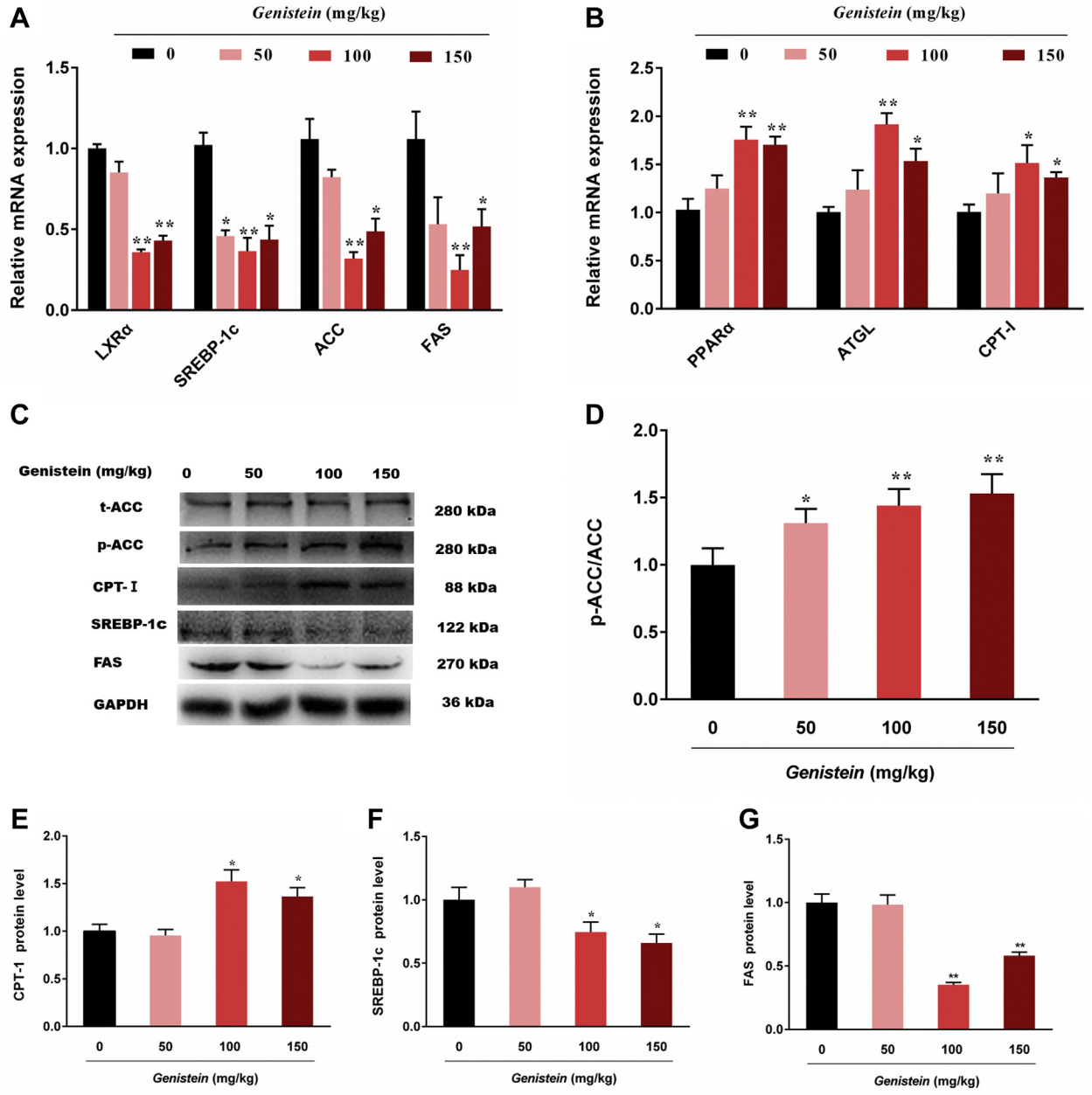

Figure 2. Effects of genistein on lipid metabolism–related factors expression level in the liver. (A) The mRNA level of lipogenesis-related factors, including liver X receptor α (LXRα), sterol regulatory element-binding protein-1c (SREBP-1c), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS); (B) The mRNA level of lipolysis-related factors, including peroxisome proliferator-activated receptor α (PPARα), fat triglyceride lipase (ATGL), and carnitine palmitoyl transferase-I (CPT-I); (C) Immunoblot of total (t)-ACC, phospho (p)-ACC, CPT-I, SREBP-1c, and FAS protein level; (D) p-ACC/ACC; (E) CPT-I protein level; (F) SREBP-1c protein level; (G) FAS protein level. Values are expressed as means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the control group.

groups compared with that of the control group ($P < 0.05$) (Figure 2E).

Impact of Genistein on the NAD$^+$/NADH Ratio and NAD$^+$ Synthesis–Related Factor Expression Levels

The NAD$^+$ concentration and NAD$^+$/NADH ratio in the liver were increased in the 100 and 150 mg/kg genistein treatment groups compared with the control group ($P < 0.05$) (Figures 3A and 3B). Compared with the control group, no significant change among the NAD$^+$ synthase, NAD 1/2/3 mRNA levels in the liver were observed in the genistein treatment groups ($P > 0.05$) (Figure 3C). However, the nicotinamide phosphoribosyl transferase (***Nampt***) mRNA level in the liver was increased in the 100 and 150 mg/kg genistein treatment groups compared with the control group ($P < 0.05$) (Figure 3D).

Impact of Genistein on the Estrogen Receptor β-Forkhead Box O1-Nampt Signaling Pathway in the Liver

No difference was observed on the *ERα* mRNA level ($P > 0.05$). The estrogen receptor β (***ERβ***) and forkhead

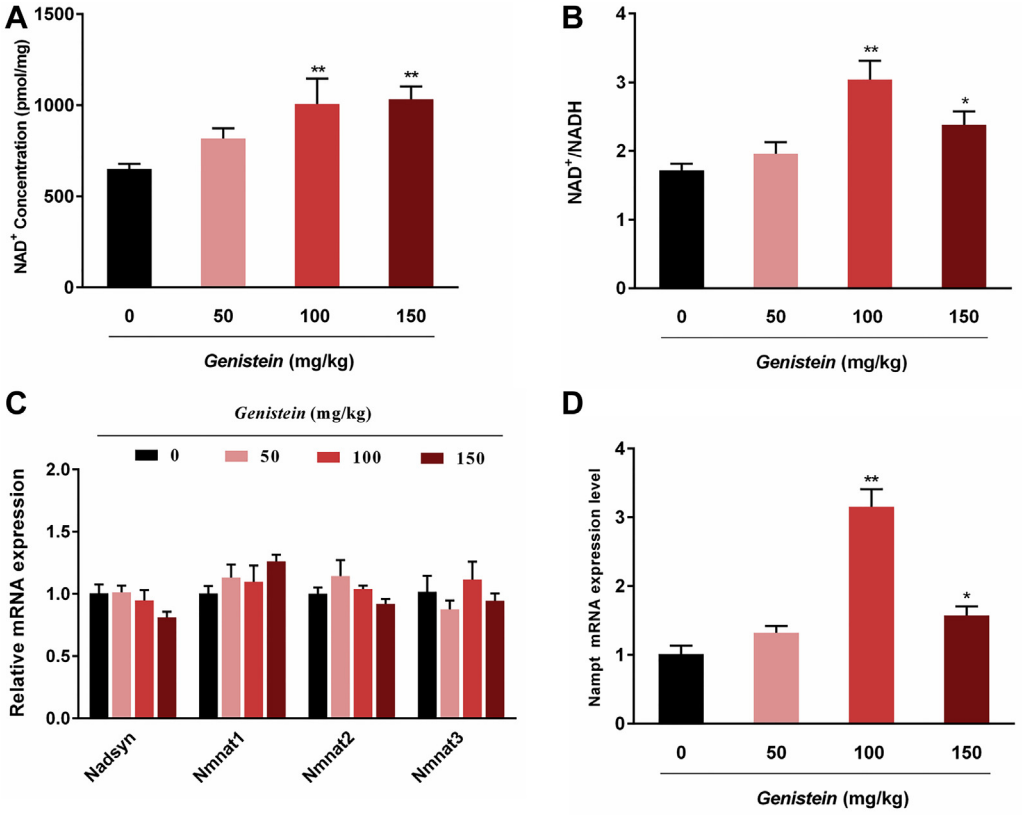

Figure 3. Effects of genistein on NAD^+ level and NAD^+ synthesis-related factors expression in the liver. (A) NAD^+ concentration; (B) NAD^+/NADH ratio; (C) The mRNA level of NAD^+ de novo biosynthesis-related genes, including NAD 1/2/3 (Nmnat1/2/3) and NAD^+ synthase (Nadsyn); (D) The mRNA level of NAD^+ salvage pathway-related factor: nicotinamide phosphoribosyl transferase (Nampt). Values are expressed as means ± SEM. $^*P < 0.05$ and $^{**}P < 0.01$, compared with the control group.

box O1 (***FOXO1***) mRNA levels were increased in genistein treatment groups compared with the control group ($P < 0.05$) (Figures 4A and 4B). Meanwhile, the protein levels of ERβ, FOXO1, and Nampt were increased in the genistein treatment groups than that of the control group ($P < 0.05$) (Figures 4C–4F).

Impact of Genistein on the Adiponectin-AMPK-SIRT1/PGC-1α Signaling Pathway in the Liver

The adiponectin concentration was increased in the 100 and 150 mg/kg genistein treatment groups compared with the control group ($P < 0.05$) (Figure 5A). No change was observed on the adiponectin receptor *1* mRNA level, but genistein treatment significantly increased the adiponectin receptor 2 mRNA level compared with the control group ($P < 0.01$) (Figure 5B). Besides, supplementation with 100 and 150 mg/kg genistein increased the *SIRT1*, *AMPKα*, and *PGC-1α* mRNA levels compared with the control group ($P < 0.05$) (Figure 6A). Meanwhile, genistein treatment increased the p-AMPK protein level ($P < 0.05$) (Figure 6D). The SIRT1 and PGC-1α protein levels were also increased in the 100 and 150 mg/kg genistein treatment groups compared with the control group ($P < 0.05$) (Figures 6C and 6E).

DISCUSSION

The chicken was favored by consumers because of its characteristics of high protein. However, consuming chickens with excessive fat deposition harms consumers' health. Diet supplements with plant extracts have been used to control lipid metabolism in poultry (Khalaji et al., 2013; Balaji et al., 2016). Genistein is one kind of plant extract and is considered to have the potential to control fat deposition or prevent obesity (Behloul and Wu, 2013). It reported that genistein could regulate lipid and energy metabolism in mice fed with a high-fat or high-fructose diet (Huang et al., 2016; Han et al., 2017). Abdominal fat is the fastest-growing adipose tissue of chickens. Thus, the abdominal fat pad is a crucial parameter to infer the fat content in broiler chickens. The present study showed that no change was observed on growth performance, but the relative weight of abdominal fat was decreased in broiler chickens with genistein treatment. In addition, our results found that genistein decreased TG content both in the serum and liver, which are consistent with those of the study by Lv et al. (2018), who reported that dietary supplementation with genistein reduced the TG content and fat deposition in chickens. Meanwhile, genistein increased the FFA and glycerol concentration both in the serum and liver, which suggested that genistein promoted the

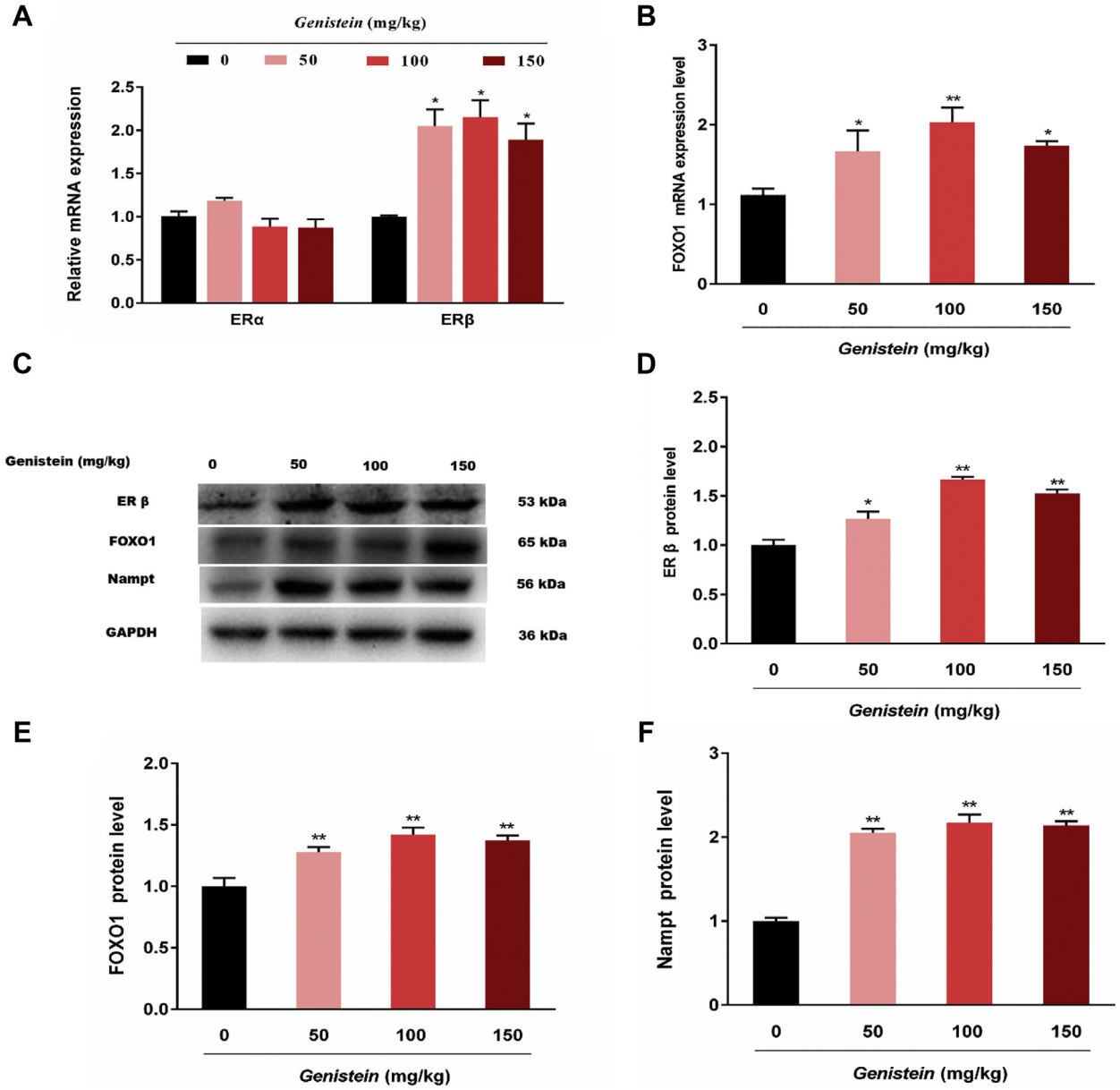

Figure 4. Effects of genistein on the ERβ-FOXO1-Nampt signaling pathway-related factors expression levels in the liver. (A) The mRNA level of estrogen receptor (ER) α and ERβ; (B) The mRNA expression level of forkhead box O1(FOXO1); (C) Immunoblot of ERβ, FOXO1, and Nampt protein level; (D) ERβ protein level; (E) FOXO1 protein level; (F) Nampt protein level. Values are expressed as means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the control group. Abbreviation: Nampt, nicotinamide phosphoribosyl transferase.

conversion of TG to glycerol and FFA (Nguyen et al., 2008). Oil red O staining is used to assess the accumulation of lipid droplets in the liver of broiler chickens, and the results showed that the quantity and total area of lipid droplets in the liver were decreased after genistein treatment. Importantly, we found that the activities of lipoprotein lipase in serum and hepatic lipase in the liver, which are essential for the utilization of lipid and facilitate TG lipolysis (He et al., 2013), were enhanced after genistein treatment. These results indicated that supplementation with genistein could reduce fat deposition in broiler chickens.

As one of the nuclear receptors, LXRα can induce fatty acid synthesis by activating SREBP-1c and subsequently stimulates the transcription of the lipogenesis-related genes, encoding FAS and ACC enzymes (Kondo et al., 2009). In this study, supplementation with genistein increased the *LXRα*, *SREBP-1c*, *ACC,* and *FAS* mRNA levels in broiler chickens. The results are consistent with the research that reported that genistein regulates lipid metabolism and cholesterol transport through regulating the expressions of lipogenesis-related factors (Arunkumar et al., 2013). In addition, a previous study reported that genistein could not only inhibit de novo lipogenesis but also enhance lipolysis in chickens (Lv et al., 2018). Therefore, we also assessed the critical hepatic lipolysis-related factors of broiler chickens in this study. We found that the *ATGL* mRNA level was increased in the liver of broiler chickens with genistein treatment, which implied that the ability

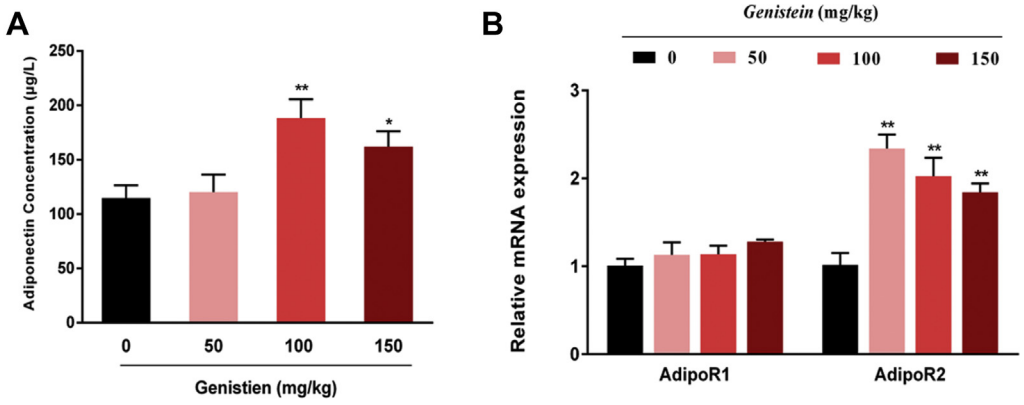

Figure 5. Effects of genistein on adiponectin concentration and adiponectin receptor expression levels. (A) Adiponectin concentration in serum; (B) Adiponectin receptor 1/2 (AdipoR 1/2) mRNA levels in the liver. Values are expressed as means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the control group.

of TG hydrolysis was enhanced (Ong et al., 2011). In addition, consistent with Liu et al. (2017), the *CPT-I* and *PPARα* mRNA levels were also enhanced after genistein treatment, which represented the ability of fatty acid β-oxidation that was improved. All aforementioned results indicated that genistein reduced fat deposition through inhibiting lipogenesis-related factor expression levels and improving lipolysis-related factor expression levels in broiler chickens.

Adenosine 5′-monophosphate-activated protein kinase and SIRT1 play an essential role in lipid metabolism (Hardie et al., 2012; Simmons et al., 2015). However, there are few research studies on how genistein activates SIRT1 and AMPK. Sirtuin1 is an NAD^+-dependent deacetylase, and research had reported that altered levels of NAD^+ or rather the ratios of NAD^+/NADH have a profound effect on SIRT1 activity (Haigis and Guarente, 2006). In our study, the NAD^+ and NAD^+/NADH ratio in the liver were increased after genistein treatment. We further detected the expression levels of crucial factors related to the NAD^+ biosynthetic pathway in the liver (Bogan and Brenner, 2008). No significant change was observed on the expression of genes involved in de novo biosynthesis of NAD^+, including

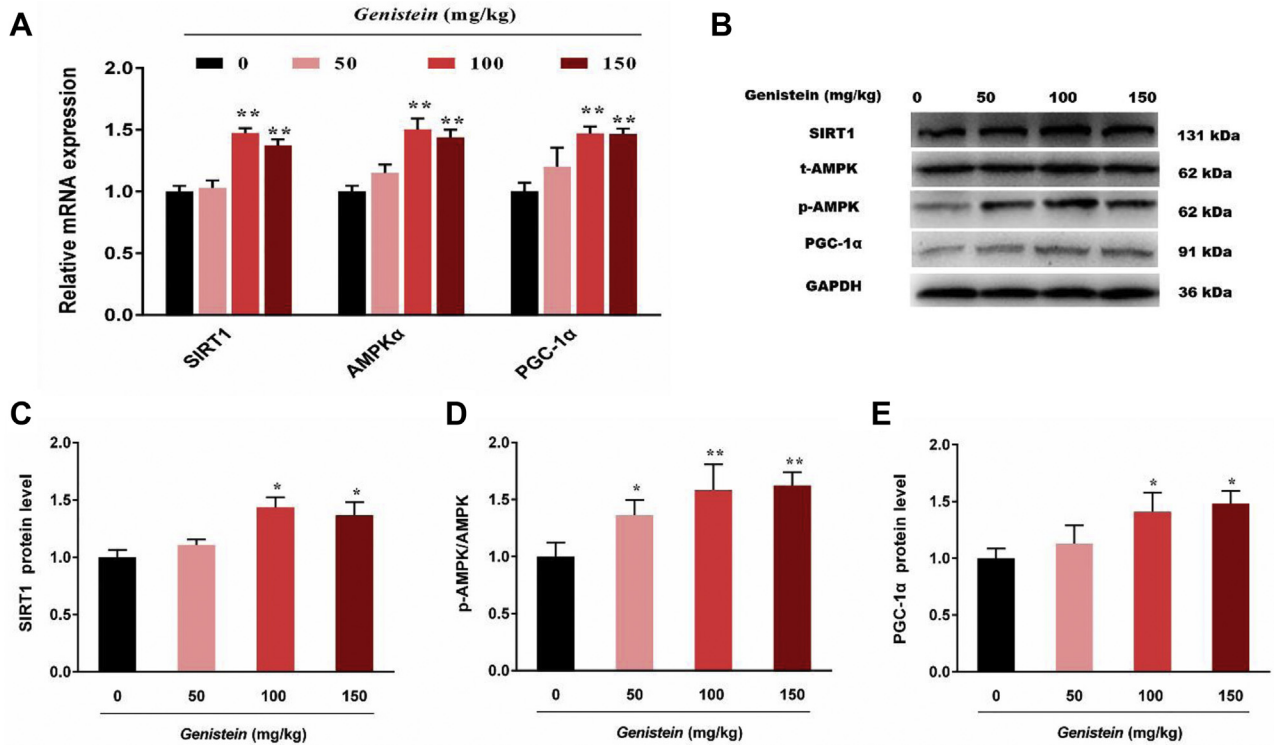

Figure 6. Effects of genistein on the AMPK-SIRT1 signaling pathway–related factors expression levels in the liver. (A) The mRNA level of SIRT1, adenosine 5′-monophosphate (AMP)-activated protein kinase α (AMPKα) and peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α); (B) Immunoblot of SIRT1, total (t)-AMPK, phospho (p)-AMPK and PGC-1α protein levels; (C) SIRT1 protein level; (D) p-AMPK/AMPK; (E) PGC-1α protein level. Values are expressed as means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the control group. Abbreviation: SIRT1, sirtuin1.

NAD^+ synthase and NAD 1/2/3. Meanwhile, the mRNA expression of *Nampt*, which is the rate-limiting factor of the NAD^+ salvage pathway, was significantly increased. The results implied that genistein improves the NAD^+ concentration through promoting the NAD^+ salvage pathway and then influences the SIRT1 activity of broiler chickens. The structure of genistein is similar to 17β-estradiol (Behloul and Wu, 2013). This particular similitude enables it to bind to estrogen receptors and thus can at least partly explain its effects. Importantly, previous studies reported that genistein preferentially activated ERβ (Chang et al., 2008). In this study, we also found that genistein treatment significantly increased the ERβ protein level in the liver of broiler chickens. Previous research has proved that ERβ is associated with the gene promoters of FOXO1 through the Krüppel-like zinc finger transcription factor 5, and the expression of ERβ could enhance the transcription of FOXO1 (Nakajima et al., 2011). Forkhead box O1 is an important regulator to maintain hepatic TG homeostasis. Tao et al. (2011) demonstrated that FOXO1 could increase the transcription of Nampt, thereby promoting the synthesis of NAD^+. In the present study, genistein increased the protein levels of ERβ, FOXO1, Nampt, and SIRT1 in the liver of chicken, which implied that the mechanism of genistein on regulating lipid metabolism might be related to the activation of ERβ-FOXO1-SIRT1 signaling pathway. Adiponectin is an important hormone secreted by adipocyte and can activate the AMPK signaling pathway *via* binding to its membrane receptors to regulate lipid metabolism in the liver (Chen et al., 2013). Although the specific mechanism of genistein regulating adiponectin is unclear, Sakaue et al. (2016) have reported that genistein could promoted adiponectin secretion in adipose-derived stem cells. Therefore, we further evaluated serum adiponectin content and the protein levels of hepatic adiponectin receptors and AMPK in broiler chickens. The results showed that the AMPK phosphorylation protein level was significantly increased in broiler chickens with genistein treatment, which is consistent with the changes in the adiponectin content and adiponectin receptor 2 mRNA expression level. These results indicated that genistein might activate AMPK by promoting adiponectin concentration in broiler chickens. In addition, a recent study reported that the paracrine acting of adiponectin could also synergize with genistein to enhance transcriptional response to ERβ signaling (Rahal and Simmen, 2011). Therefore, we speculated that the activation of the ERβ-FOXO1-SIRT1 signaling pathway induced by genistein in broiler chickens might also be related to the paracrine acting of adiponectin. Certainly, further investigation should be focused at the cellular level to support this conclusion.

The homeostasis of lipid in the body is related to the balance of lipogenesis and lipolysis (Saponaro et al., 2015). As the crucial factors of lipid metabolism, previous research studies have reported that there exists an interdependence between AMPK and SIRT1 and can both regulate each other and share many common target factors (Cantó et al., 2010; Ruderman et al., 2010). In this study, as the downstream factors of AMPK and SIRT1, the SREBP-1c and FAS protein levels were decreased, whereas the p-ACC protein level was increased after genistein treatment. Combined with the increase of p-AMPKα and SIRT1 protein levels, the results indicated that the activation of AMPK-SIRT1 might be one of the mechanisms by which genistein regulates the lipogenesis-related factors expression levels in broiler chickens. In addition, PGC-1α is a transcriptional coactivator and can be activated by SIRT1 and AMPK, which subsequently promotes mitochondrial biogenesis and energy metabolism (Cantó and Auwerx, 2009). Song et al. (2004) suggested that the increased expression of PGC-1α in the liver could stimulate fatty acid oxidation *via* improving CPT-I expression. In our study, genistein also enhanced the protein expression levels of PGC-1α and CPT-I in broiler chickens, which suggested that the fatty acid β-oxidation were promoted in the liver of broiler chickens. These results implied the regulation of genistein on fat deposition in broiler chickens might be because of the activation of the AMPK-SIRT1/PGC-1α signaling pathway.

CONCLUSION

Our data showed that dietary genistein supplementation reduced the abdominal fat deposition in broiler chickens. We speculated that the effects of genistein regulating fat accumulation in broiler chickens might be owing to the activation of the AMPK-SIRT1/PGC-1α signaling pathways. Furthermore, the mechanism of genistein to activate the signaling pathways might be achieved by its direct effect on improving the secretion of adiponectin or its indirect effect on the upregulation of ERβ expression through the paracrine acting of adiponectin. The specific regulation mechanism of genistein on lipid metabolism remains to be further verified at the cellular level. The results presented here are partially to increase our understanding of the mechanisms underlying the fat-reduction action of genistein and further support it as a nutritional supplement to control fat deposition or lipid metabolism–related diseases in poultry.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.10.013.

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