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The effect of equol injection *in ovo* on lipid metabolism and hepatic lipogenic gene expression in broilers

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This study investigated the effects of in ovo *administration of equol (Eq) on post-hatch growth and hepatic lipid metabolism in broiler chickens. Fertilized eggs (146 eggs/group) were injected with 0 μg (control, Con), 20 μg (low dose, L) and 100 μg (high dose, H) Eq in the albumen on the 7th day of incubation. Except a trend increase in the weight of total fat (*$P = 0.09$*), Eq had no effect on growth or liver weight in broilers at 49 days of age. Males presented higher liver and BWs and lower total fat and relative liver weights than females (*$P < 0.01$*). However, there were no significant effects of Eq or Eq–gender interactions on growth performance or tissues weight (*$P > 0.05$*). With respect to lipid parameters in the serum, the results showed that female broilers presented higher triacyglycerol (TG) and low-density lipoprotein cholesterol concentrations than males, whereas there was no gender difference in serum total cholesterol (TC) or high-density lipoprotein cholesterol (HDLC) concentration (*$P > 0.05$*). Eq administration significantly decreased serum TG and TC but increased HDLC concentrations in serum of broilers at 49 days of age (*$P < 0.05$*), whereas there were no interactions between gender and Eq (*$P > 0.05$*). To elucidate the mechanism behind the significant changes of serum TG and TC levels, the expression of genes involved in lipid metabolism in the liver was investigated in female chickens using reverse transcription-PCR. Carnitine palmitoyl transferase I (CPTI) messenger RNA (mRNA) was significantly upregulated by 20 and 100 μg Eq (*$P < 0.05$*). High-dose Eq significantly decreased fatty acid synthase (FAS) and enhanced cholesterol-7alpha-hydroxylase (CYP7A1) mRNA levels in the liver (*$P < 0.05$*). Eq had no significant effects on acetyl-CoA carboxylase, sterol regulatory element binding protein-1c, malic enzyme, low-density lipoprotein receptor or 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA in the liver (*$P > 0.05$*). These results in female broilers suggest that Eq decreased blood TG by upregulating CPTI and downregulating FAS mRNA expression in the liver, and that high serum cholesterol levels stimulated CYP7A1 gene transcription in the liver.*

Keywords: equol, *in ovo* injection, lipid metabolism, liver, broiler

Implications

Numerous studies have demonstrated that isoflavones have beneficial effects on lowering serum lipids and decreasing body fat deposition (Bhathena and Velasquez, 2002). Cederroth *et al.* (2007) reported that male CD-1 mice fed from conception to adulthood a high soy-containing diet significantly increased energy expenditure and decreased adiposity (Cederroth *et al.*, 2007). Daidzein is a major isoflavonic phytoestrogen and has a variety of biological functions (Cassidy, 2003; Hwang *et al.*, 2006). As a metabolite of daidzein, equol (Eq) possesses more biological activities than its precursor (Matthies *et al.*, 2008). Eq exists in hens' blood and accumulates into eggs, preferentially into egg yolk (Saitoh *et al.*, 2001). However, whether Eq existing in eggs would affect lipid metabolism of the hatchlings is unclear. Therefore, the aim of this study was to investigate the effects of Eq directly injected *in ovo* on lipid metabolism and the related gene transcription in the liver of broilers.

Introduction

Phytoestrogens are a large group of plant-derived compounds, and they have attracted considerable interest in both medical and agricultural scientific research for decades because of their wide array of bioactive functions. Phytoestrogens include isoflavonoids, lignans, coumestanes, stilbenes and flavonoids (Price and Fenwick, 1985). Numerous studies in humans and rodents have suggested that, like estrogens,

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dietary phytoestrogens and their metabolites play a beneficial role in reducing obesity and body mass index (Bhathena and Velasquez, 2002). In contrast, however, other studies have reported no beneficial effects of these chemicals on fat mass and serum lipid profiles (Yeung and Yu, 2003; Anderson *et al.*, 2007). These discrepancies are probably related to differences among the experimental designs and protocols (e.g. route of administration, composition, dose and duration), as well as the capacity of individuals to produce equol (Eq; Setchell, 2001).

Biotransformation is an important factor in regulating the biological activity of dietary isoflavones. Daidzein is known to be converted into Eq or O-desmethylangolen sin (O-DMA) by bacterial enzymes in the intestine (Matthies *et al.*, 2008). Eq is absorbed more efficiently and has higher estrogenic activities than its precursor daidzein. Eq has been found in the blood of hens and accumulates predominantly in eggs (Saitoh *et al.*, 2001 and 2004). Nevertheless, the effect of Eq in eggs on the growth and lipid metabolism of post-hatch chickens has not yet been elucidated. The fertilized egg provides a good model because it is enclosed by an eggshell and is relatively unaffected by external factors.

The liver is known to be the primary site of fatty acid synthesis in birds (Xu *et al.*, 2003), and most of the body's endogenous lipids are hepatic in origin (O'Hea and Leveille, 1969). As in mammals, enzymes in the avian liver control sterol and lipid metabolism, and the levels of mRNA expression of lipogenic genes have been reported to parallel changes in enzyme activity, such as fatty acid synthase (FAS; Back *et al.*, 1986). In contrast to the large number of studies of hepatic lipid metabolism and the effects of isoflavones on lipogenic gene expression in rodents and humans, little is known about the effects of isoflavones on hepatic lipid metabolism in poultry, and especially about the effects of Eq on lipid liver metabolism in broiler chickens. Therefore, the aim of the present study was to investigate the effects of *in ovo* Eq administration on growth and lipid metabolism in post-hatch broilers. The study focused on changes in gene expression in the liver, with particular emphasis on the genes involved in fatty acid synthesis and cholesterol metabolism, including sterol regulatory element binding protein-1c (SREBP-1c), acetyl-CoA carboxylase (ACC-α), FAS, carnitine palmitoyl transferase I (CPTI), malic enzyme (ME), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), low-density lipoprotein receptor (LDLR) and cholesterol-7alpha-hydroxylase (CYP7A1).

Material and methods

Animals

Animals and Experimental Protocol have been well described previously (Wei *et al.*, 2011). In brief, on the 7th day of embryonic age, fertile eggs (Chinese local yellow broiler) were randomly allotted to three groups (146 eggs/group): 0 μg (control, Con), 20 μg (low dose, L) and 100 μg (high dose, H) Eq dissolved in 100 μl mixture of white mineral oil and ethanol were injected into the albumen of the fertile eggs, respectively. After hatching, broilers were raised in three pens (one pen/group) and split into 12 pens (four pens/group, 30 chickens/pen) at 21 days of age. Chickens were raised under the same standard conditions with free access to water and fed until 49 days of age.

At 49 days of age, birds were deprived of feed for 12 h and weighed just before slaughter; 20 broilers were taken randomly from each group and slaughtered in the morning by cervical dislocation for carcass analysis, blood and tissue sampling. Sera were stored at -20°C until triacyglycerol (TG) analysis. The liver was immediately removed from the body and weighed, and the same portion was sampled and snap-frozen in liquid nitrogen and stored at -70°C before analysis. Subcutaneous fat and abdominal fat were collected and weighed.

Animal care and use as described above was approved by the Institutional Animal Care and Use Committee of Nanjing Agricultural University.

Materials

Eq (purity $\geqslant$99%, Catalog No. E-5880) was bought from Shanghai EQUL company (Shanghai, China) and synthesized from LC laboratories in the United States of America (Woburn, MA, USA).

Measurement of lipid parameters

Serum total cholesterol (TC) and TG concentrations were determined using enzymatic kits based on the 'CHOD-PAP' and 'GPO-PAP' reactions, respectively (Watson *et al.*, 1995). High-density lipoprotein cholesterol (HDLC) values were measured after magnesium chloride–heparin sulfate precipitation of apo-B-associated lipoproteins (low-density lipoprotein – LDL and very-low-density lipoprotein – VLDL). The relevant reagents were purchased from the Nanjing Jiancheng Bioengineering Institute (NJBI, China). The optical density of the samples was measured using a spectrophotometer three times at a wavelength of 546 nm (Biotech, Foster, USA).

Measurement of gene expression

RNA extraction. Total RNA was extracted from 100 mg of liver (10 for each group) using TRIZOL reagent (Takara, Kyoto, Japan) according to the manufacturer's protocols. In brief, 100 mg liver tissue was disrupted and homogenized immediately using 0.5 ml of 100% isopropanol and 1 ml of TRIZOL reagent. After incubation for 10 min at room temperature, the lysate was centrifuged at 12 000 $\times$ **g**/min for 10 min at 4°C. Subsequently, the RNA was washed with 1 ml of 75% ethanol and was dissolved in RNase-free water. The RNA concentration was then quantified by measuring the absorbance at 260 nm in a photometer (Eppendorf Biophotometer, Hamburg, Germany). Ratios of absorption (260/280 nm) of all preparations were between 1.8 and 2.0. Aliquots of RNA samples were subjected to electrophoresis with 1.4% agarose–formaldehyde gels stained with ethidium bromide to verify their integrity.

Real-time quantitative reverse transcription (RT)-PCR. Reverse transcription was performed using the total RNA (2 μg)

described above in a final volume of 25 μl containing 1 × RT-buffer, 100 U Moloney Murine Leukemia Virus reverse transcriptase (M-MLV; Promega, Madison, USA), 8 U RNase inhibitor (Promega, USA), 5.3 μM random hexamer primers and 0.8 mM dNTP (TaKaRa, Japan). After incubation at 37°C for 1 h, the reaction was terminated by heating at 95°C for 5 min and quickly cooling on ice. Real-time PCR was performed in Mx3000P (Stratagene, USA). Mock RT and No Template Controls were set to monitor the possible contamination of genomic DNA both at RT and PCR. The pooled sample obtained by mixing equal quantity of total RT products (cDNA) from all the samples was used for optimizing the PCR condition and tailoring the standard curves for each target gene, and melting curves were performed to insure a single specific PCR product for each gene. A volume of 2 μl of 16-fold dilution of RT product was used for PCR in a final volume of 25 μl containing 12.5 μl SYBR Green Real-time PCR Master Mix (TOYOBO Ltd, Japan) and 0.4 to 0.8 μM of each forward and reverse primers for SREBP-1c, ACC-α, FAS, CPTI, ME, HMGR, LDLR and CYP7A1 genes (the information of primers was shown in Table 1). Chicken β-actin mRNA was used as a reference gene for normalization purpose. The following PCR protocols were initial denaturation (1 min at 95°C), followed by a three-step amplification program (20 s at 95°C, 20 to 30 s at 60 to 64°C, 30 s at 72°C), which was repeated 45 times. The PCR products for each gene were sent to Haojia Biotech Ltd, China for sequencing to verify the specificity. The reported sequences exactly matched those published in GenBank. The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real-time RT-PCR data (Livak and Schmittgen, 2001). All the samples were included in the same run of RT-PCR and repeated in triplicate.

Statistical analysis

The results are expressed as mean ± s.e.m. Data were analyzed by ANOVA using the General Linear Models of SPSS 11.0 for Windows (StatSoft Inc., USA). Growth performance, tissues weight and serum lipid parameters were analyzed for differences due to gender, Eq and gender–Eq interactions. The multiple comparisons of the interactive means were performed using the Tukey correction. The effects of Eq administration on the hepatic metabolism gene expression data were analyzed by one-way ANOVA followed by least significant difference test *post hoc* analysis. Data were considered statistically significant when $P < 0.05$.

Results

Growth performance and weight of tissues

The results showed that males presented higher liver weight (24.59 ± 0.80 for males *v.* 17.29 ± 0.43 for females) and BW (966.40 ± 14.40 for males *v.* 642.18 ± 12.36 for females) and lower total fat (9.656 ± 0.80 for males *v.* 13.58 ± 1.17 for females) and relative liver weights (2.54 ± 0.03 for males *v.* 2.70 ± 0.02 for females) compared with females ($P < 0.01$; Table 2). Except a trend for an increase in the weight of total fat ($P = 0.09$), there was no significant effect of Eq treatment on BW, liver weight or the relative weight of liver to BW. In addition, there was no significant effect of Eq and Eq–gender interactions on growth performance or tissues weight ($P > 0.05$).

Serum lipid metabolic parameters

The results of serum lipid metabolic parameters of broilers are presented in Table 3.

Table 1 *Summary of the oligonucleotide PCR primer sets*

Gene	GenBank accession number	Primer sequence (5′-3′)	Orientation	Product size (bp)
ACC-α	X03805	GATATTGATGATCGGCTTAG	Forward	418
		GTGCTCCATTCCTTTGTT	Reverse	
FAS	AB075215	TTACTTCAACACATCCTACACC	Forward	346
		TCATAATCTCTTCCCATCTTCA	Reverse	
SREBP-1c	BQ037565	GTACTTCAGTGCTTCGGATGTG	Forward	397
		CTTCTTCAGAGTTGGAGGTGCT	Reverse	
CPTI	AY675193	CAATGCGGTACTCCCTGAAA	Forward	337
		CATTATTGGTCCACGCCCTC	Reverse	
ME	AF408407	AGCATTACGGTTTAGCATTTCGG	Forward	239
		CAGGTAGGCACTCATAAGGTTTC	Reverse	
HMGR	AB109635	TTGGATAGAGGGAAGAGGGAAG	Forward	137
		CCATAGCAGAACCCACCAGA	Reverse	
CYP7A1	AB109636	CATTCTGTTGCCAGGTGATGTT	Forward	106
		GCTCTCTCTGTTTCCCGCTTT	Reverse	
LDLR	NM_204452	CCACCATTTGGCAGAGGAA	Forward	86
		ACCGCAGTCAGACCAGAAGAG	Reverse	
β-actin	L08165	ACGTCGCACTGGATTTCGAG	Forward	282
		TGTCAGCAATGCCAGGGTAC	Reverse	

ACC-α = acetyl CoA carboxylase; FAS = fatty acid synthase; SREBP-1c = sterol regulatory element binding protein-1c; CPTI = carnitine palmitoyl transferase I; ME = malic enzyme; HMGR = 3-hydroxy-3-methylglutaryl coenzyme A reductase; CYP7A1 = cholesterol-7alpha-hydroxylase; LDLR = low-density lipoprotein receptor.

Table 2 *Effect of Eq injected* in ovo *on growth performance of male and female broilers at 49 days of age*

	Male broilers			Female broilers			*P*-value		
Groups	Con	L	H	Con	L	H	Gender	Treatment	Gender × treatment
BW (g)	939.57 ± 25.46	991.74 ± 24.24	965.16 ± 24.86	622.88 ± 14.11	667.86 ± 28.02	632.95 ± 9.26	<0.001	0.319	0.795
LW (g)	24.17 ± 0.66	25.15 ± 0.36	24.52 ± 0.94	16.45 ± 0.43	18.18 ± 0.92	17.24 ± 0.78	<0.001	0.436	0.172
LW/BW[1] (%)	2.58 ± 0.05	2.49 ± 0.06	2.61 ± 0.05	2.68 ± 0.06	2.71 ± 0.06	2.77 ± 0.08	<0.001	0.129	0.624
FW[2] (g)	8.93 ± 1.42	8.77 ± 0.97	11.48 ± 1.68	10.74 ± 1.14	14.93 ± 2.28	15.82 ± 2.61	<0.001	0.088	0.102

Eq = equol; BW = body weight; LW = liver weight; FW = fat weight.
Values are means ± s.e.m. Con, L and H stand for control, low and high dosage of Eq injected into the albumen of the fertile eggs on the 7th day of embryonic age, respectively.
[1]LW/BW: the relative weight of liver to BW.
[2]FW containing subcutaneous and abdominal fat tissues.

Table 3 *Effect of Eq injected* in ovo *on TG, TC, LDLC and HDLC in serum of male and female broilers at 49 days of age*

	Male broilers			Female broilers			*P*-value		
Groups	Con	L	H	Con	L	H	Gender	Treatment	Gender × treatment
TG (mmol/l)	0.90 ± 0.07	0.80 ± 0.03	0.81 ± 0.04	1.72 ± 0.17	1.44 ± 0.19	1.05 ± 0.08	0.001	0.025	0.119
TC (mmol/l)	3.23 ± 0.10	3.42 ± 0.09	3.49 ± 0.11	2.79 ± 0.11	3.25 ± 0.06	3.32 ± 0.21	0.085	0.043	0.889
LDLC (mmol/l)	0.62 ± 0.09	0.86 ± 0.04	0.83 ± 0.06	0.73 ± 0.12	0.92 ± 0.22	0.93 ± 0.23	0.034	0.948	0.907
HDLC (mmol/l)	2.03 ± 0.09	2.24 ± 0.09	2.28 ± 0.07	1.79 ± 0.11	2.31 ± 0.13	2.36 ± 0.17	0.202	0.001	0.203

Eq = equol; TG = triacyglycerol; TC = total cholesterol; LDLC = serum low-density lipoprotein cholesterol; HDLC = high-density lipoprotein cholesterol.
Values are means ± s.e.m. Con, L and H stand for control, low and high dosage of Eq injected into the albumen of the fertile eggs on the 7th day of embryonic age, respectively.

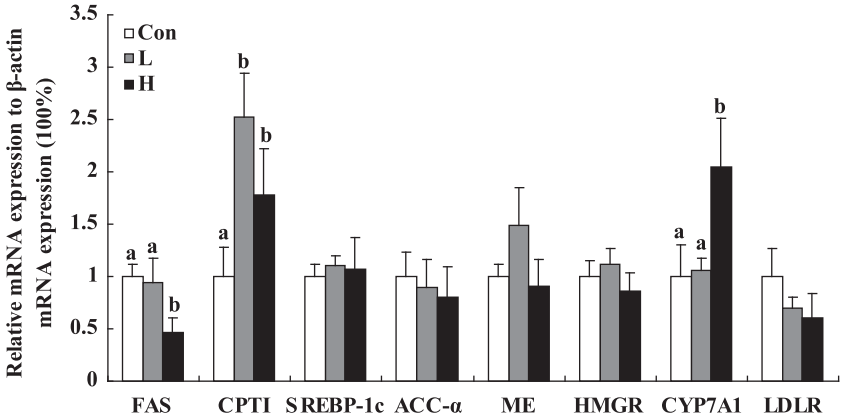

Figure 1 Effects of Eq on the mRNA expression of FAS, CPTI, CYP7A1, SREBP-1c, ACC-α, ME, HMGR and LDLR genes in liver of female broilers. Con, L and H stand for control, low and high dosage of Eq injected into the albumen of the fertile eggs on 7th day of embryonic age, respectively. Data are means ± s.e.m. from three different experiments in which each treatment was performed in triplicate within the experiment. Mean values without common superscript (a and b) differ significantly among the Con, L and H groups ($P < 0.05$, $n = 10$). FAS = fatty acid synthase; CPTI = carnitine palmitoyl transferase I; CYP7A1 = cholesterol-7alpha-hydroxylase; SREBP-1c = sterol regulatory element binding protein-1c; ACC-α = acetyl-CoA carboxylase; ME = malic enzyme; HMGR = 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDLR = low-density lipoprotein receptor.

Male broilers presented significantly lower serum TG concentration than females (0.84 ± 0.30 for males *v.* 1.44 ± 0.10 for females; $P < 0.05$). Eq showed a significant effect on decreasing serum TG concentration of broilers at 49 days of age (0.97 ± 0.07 in H group and 1.19 ± 0.12 in L group *v.* 1.32 ± 0.12 in Con group; $P < 0.05$), whereas there were no interactions between gender and Eq ($P > 0.05$). Eq significantly increased serum TC (3.41 ± 0.07 in H group and 3.34 ± 0.05 in L group *v.* 3.09 ± 0.07 in Con group) and HDLC concentrations (2.43 ± 0.11 in H group and 2.22 ± 0.07 in L group *v.* 1.90 ± 0.11 in Con group; $P < 0.05$), whereas Eq did not affect serum low-density lipoprotein cholesterol (LDLC) concentration ($P > 0.05$). Except a significant gender difference of serum LDLC concentration (0.77 ± 0.04 for males *v.* 0.87 ± 0.11 for females), there was no gender difference in serum TC (3.38 ± 0.06 for males *v.* 3.17 ± 0.99 for females) or HDLC concentrations (2.19 ± 0.05 for males *v.* 2.14 ± 0.09 for females; $P > 0.05$). There were no gender–Eq interactions on the level of TC, HDLC or LDLC concentrations in the serum of broilers at 49 days of age ($P > 0.05$).

Hepatic lipid metabolism gene expression

As shown in Figure 1, the gene expression of FAS in the liver of female broilers at 49 days of age was significantly downregulated by high-dose Eq ($P < 0.05$), whereas hepatic CPTI mRNA expression was significantly upregulated by both low- and high-dose Eq treatment ($P < 0.05$). High-dose Eq significantly increased hepatic CPY7A1 mRNA expression ($P < 0.05$). However, the expression levels of SREBP-1c, ACC-α, ME, LDLR and HMGR genes were not affected by Eq treatment ($P > 0.05$).

Discussion

Our results showed that except a trend increase in the weight of total fat, there was no significant effect of Eq treatment on BW, liver weight or the relative weight of liver to BW ratio, whereas there was a significant gender effect on BW and the tissues weight of broilers at 7 weeks of age. Eq showed significant effects on serum TG, TC and HDLC concentrations of broilers at 49 days of age, whereas there were no interactions between gender and Eq. On the basis of the results of hepatic genes expression measured in female chickens by real-time PCR, the changes in sterol and lipid metabolic parameters in the serum were accompanied by significant increases in CPTI and CYP7A1 1 gene expression and decreased FAS mRNA transcription in the liver.

Numerous studies have demonstrated that isoflavones have beneficial effects on lowering serum lipids and decreasing body fat deposition (Bhathena and Velasquez, 2002). Supplementation of the soy protein diet with isoflavone in rats has been reported to dose-dependently decrease serum TG concentration (Takahashi *et al.*, 2009). Several studies have also reported that soy protein rich in isoflavones reduces fat-pad mass in rats, accompanied by decreased serum TG concentrations and cholesterol levels (Akahoshi *et al.*, 2004; Torres *et al.*, 2006). It has been suggested that isoflavones are the principal physiologically active components responsible for the lipid-lowering effects of soy (Bhathena and Velasquez, 2002). The results of the present study showed that serum TG concentrations in broilers decreased following *in ovo* Eq injection during the early embryonic period, without changes in BW or fat deposition. These results indicate that isoflavones and the metabolite Eq have lipid-lowering effects in mammals, as well as in chickens. This was in contrast to the results in chickens fed estradiol, which led to a marked elevation in plasma lipids, with the greatest increases in TG and LDLC concentrations (Hermier *et al.*, 1989; Park and Cho, 1990). Estrogen use in mammals, however, has been reported to decrease TG and LDLC levels and to increase HDLC levels (Haffner and Valdez, 1995). This discrepancy could be because of the different reproductive characteristics of birds and mammals. In addition, the different effects of estradiol and phytoestrogens in chickens suggest that phytoestrogens regulate lipid metabolism through

anti-estrogenic bioactivity (Hwang *et al*., 2006), or through other non-estrogen receptor pathways, such as via adipogenic transcription factors, as has been suggested in rodents (Ørgaard and Jensen, 2008). With respect to the cholesterol metabolism, dietary supplementation with soy isoflavones in quail significantly reduced the levels of cholesterol in the liver and muscles (Yilmaz *et al*., 2008). Blair *et al.* (2002) reported that soy or isoflavone extract fed to female hamsters resulted in a significant increase in plasma HDLC concentrations, but no changes in plasma LDLC and VLDLC concentrations, compared with those fed the control diet (Blair *et al*., 2002). In addition, it has been reported that the intake of soy isoflavones appeared to improve serum cholesterol levels in female, but not in male mice, rats and hamsters (Kishida *et al*., 2006; Mezei *et al*., 2006). In this study, TC and HDLC concentrations in broilers were significantly increased by Eq treatment; however, there was no significant interaction between sex and Eq treatment.

In the light of a significant decrease in serum TG concentration induced by Eq injection *in ovo* on the 7th day of incubation, we speculate that the changes in TG levels might generate the programming effects on reproduction, especially in female broilers, because egg production is an energy-intensive process, requiring a large increase in supplying of lipids (TG) to support the demands of new yolk formation. On the basis of this consideration, the mechanism behind the changes of TG concentration in serum of female broilers was elucidated in this study. The biological effects of soy phytochemicals on lipid metabolism are likely to be regulated through changes in gene expression (Torres *et al*., 2006). Soy protein diets in rodents suppressed the expression of hepatic lipogenic enzymes and lowered the expression of SREBP-1, FAS and ME in the liver (Iritani *et al*., 1996; Ascencio *et al*., 2004). The liver in birds is known to be the main site of fatty acid synthesis (Xu *et al*., 2003), and liver fatty acid metabolism has been recognized as the main source of variation in fat deposition and blood TG concentrations in poultry (O'Hea and Leveille, 1969). Previous studies have shown that SREBP-1c can directly stimulate the transcription of genes encoding ACC, the rate-limiting enzyme in fatty acid synthesis (Stoeckman and Towle, 2002; Yin *et al*., 2002), making it a good candidate for a common regulator of the lipogenic genes expressed in avian liver. However, the results of the present study revealed no evidence that either SREBP-1c or ACC genes was directly responsible for the effect of Eq on lipid synthesis at the transcriptional level.

CPTI catalyzes the formation of long-chain acylcarnitine from activated fatty acids and free carnitine, thus controlling the rate of β-oxidation and regulating the deposition or oxidation of fatty acids (Zammit, 1999). FAS is a key enzyme in fatty acid synthesis that catalyzes the synthesis of long-chain fatty acids through the condensation of acetyl-CoA and malonyl-CoA (Back *et al*., 1986). In rats, dietary soybean protein enriched with isoflavones reduced the concentrations of TG in plasma and liver, which is accompanied by decreases with lipogenic enzyme activities in the liver, including ME, FAS and ACC enzymes (Iritani *et al*., 1986). Morifuji *et al.* (2006) reported that feeding soy protein to rats decreased hepatic TG levels and epididymal fat mass, and increased hepatic CPTI activity and mRNA levels in the liver (Morifuji *et al*., 2006). Similarly, when compared with casein, soy protein upregulated CPT activity to accelerate hepatic fatty acid oxidation in rats (Banz *et al*., 2004). Expressions of the hepatic SREBP-1 and its target gene FAS were also significantly reduced by soy protein feeding in obese Zucker fa/fa rats (Tovar *et al*., 2005). In the current study, the expression of CPTI gene in the liver of female broilers was significantly upregulated by *in ovo* Eq injection, which paralleled the significant reduction in hepatic FAS mRNA expression. Although mRNA levels are not always directly correlated with the activity of the encoded functional protein, there is evidence to suggest that the transcriptional levels of several hepatic lipogenic genes, including FAS, ME and CPT genes, reflect the corresponding enzyme activity levels (Banz *et al*., 2004; Torres *et al*., 2006). Our results indicated that *in ovo* Eq injection reduced serum TG levels in female broilers by suppressing fatty acid synthesis and accelerating β-oxidation in the liver.

The liver is an important site of both cholesterol anabolism and catabolism, under the control of the HMGR and CYP7A1 enzymes, respectively. CYP7A1 regulates the removal of substantial amounts of cholesterol from the body by its excretion into bile. Moreover, the liver is a key element in the control of plasma cholesterol regulation by hepatic LDLRs (Spady *et al*., 1998; Sato *et al*., 2003). Expressions of both HMGR and CYP7A1 genes in the liver in hens are increased during sexual maturation to support the enhanced cholesterol metabolism required for egg production (Sato *et al*., 2003). However, these two genes were not modulated by dietary estradiol supplementation, indicating that estradiol does not affect cholesterol metabolism at the transcriptional level during egg production (Sato *et al*., 2003). However, there is evidence to suggest that the gene expression of hepatic HMGR and CYP7A1 is regulated by cholesterol in the diet and by HDLC and LDLC in the plasma, indicative of feedback regulation (Goldstein and Brown, 2009). HMGR mRNA expression in the liver in hens was significantly reduced by feeding a 3% cholesterol-supplemented diet, whereas CYP7A1 mRNA expression levels were markedly increased compared with those in hens fed the control feed (Sato *et al*., 2003), as seen in mammals. In the present study, *in ovo* Eq administration significantly increased serum TC and HDLC levels in female broilers and CYP7A1 mRNA expression in the liver, without affecting serum LDLC concentrations or hepatic LDLR and HMGR gene expression. The hepatic CYP7A1 gene was significantly upregulated by high dosage of Eq treatment, possibly as the result of feedback regulation by higher serum levels of TC in female broilers. However, the mechanism underlying the changes of TC in serum still needs further study.

In conclusion, the present study revealed that Eq decreased blood TG in broilers. For female broilers, the decrease in serum TG concentration induced by Eq injection *in ovo* might be caused by upregulating CPTI and downregulating FAS mRNA expression in the liver, and that high serum cholesterol levels stimulated the increase in hepatic CYP7A1 gene expression. The mechanism behind the long-term effects of Eq on lipid

and cholesterol metabolism, as well as the relevant genes expression, may be associated with some kinds of programming process involved in chicken embryonic development; however, the exact mechanisms still need further study.

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