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ORIGINAL ARTICLE

Effects of In Ovo Administration of DHEA on Lipid Metabolism and Hepatic Lipogenetic Genes Expression in Broiler Chickens During Embryonic Development

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Abstract In order to study the mechanism of DHEA (Dehydroepiandrosterone) in reducing fat in broiler chickens during embryonic development, fertilized eggs were administrated with DHEA before incubation and its effect on lipid metabolism and expression of hepatic lipogenetic genes was investigated. The mRNA levels of acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), malic enzyme (ME), apolipoprotein B100 (apoB100) and sterol regulator element binding protein-1c (SREBP-1c) were determined using real time quantitative PCR. Samples of livers were collected from the chickens on days 9, 14, and 19 of embryonic development as well as at hatching. Blood samples were extracted on days 14, 19 of incubation and at hatching. The results showed that DHEA decreased the concentration of triacyglycerol in the blood and the content in liver, and the mRNA levels of ACC, FAS, ME, SREBP-1c and apoB. This suggested that DHEA decreased the expression of hepatic lipogenetic genes and suppressed triglycerols transport, by which it reduced the deposition of fat in adipose tissue in broiler chickens during embryonic development and hatching.

Keywords Lipid metabolism · mRNA · Liver · Broiler · DHEA

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Introduction

In the last few decades, the aim of poultry production in many countries has been to increase the growth rates, but excessive fat deposition in the abdomen has been neglected. Fatness needs to be controlled, due to its negative effect on productivity. In meat-type chickens, excessive adipose tissue reduces both feed efficiency during rearing and the yield of lean meat after processing. In avian species, the liver is the main site of de novo fatty acid synthesis and accounts for 95% in young chicks [1, 2]. In consequence, most of the endogenous body lipids are of hepatic origin [3] and the development of adipose tissue depends on the availability of plasma triglycerides that are hydrolyzed prior to their utilization by adipocytes. Triglycerides are supplied specifically to adipocytes by specific lipoprotein classes: very low density lipoproteins (VLDL) transport de novo synthesized hepatic lipids [4, 5]. Thus a higher rate of triglyceride synthesis and transport from the liver is responsible for the higher weight of abdominal fat in chickens.

According to previous studies, differences in the degree of obesity are due to various steps of lipid metabolism, among which liver fatty acid metabolism has been considered as the main source of variability [6–9]. These results prompted researches into gene expression in the liver, especially those genes involved in fatty acid synthesis and secretion [10 11]. Accordingly, the present study focused on the expression of lipogenetic genes in liver. Acetyl-CoA carboxylase (ACC), the rate-limiting enzyme in fatty acid synthesis, catalyzes the carboxylation of acetyl-CoA to malonyl-CoA [12]. Fatty acid synthase (FAS) is a key enzyme in fatty acid synthesis that catalyzes the synthesis of long-chain fatty acid through the condensation of acetyl-CoA and malonyl-CoA in a

complex seven-step reaction [13]. Malic enzyme catalyzes the oxidative decarboxylation of malate to pyruvate and CO_2 simultaneously generating NADPH from $NADP^+$. In the avian liver, most of the NADPH used by fatty acid for catalyzing the synthesis of palmitate is generated by the malic enzyme [14]. The availability of apoB plays a major role in determining the capacity of hepatocytes to assemble and secrete VLDL [15, 16]. SREBP (Sterol response element binding protein) is among many potential regulators. These transcription factors of the leucine zipper family have been described as regulators of biosynthesis of cholesterol and fatty acids in the liver [17]. SREBP-1c is preferentially involved in the activation of genes that control the synthesis of fatty acid [18, 19]. The gene for SREBP-1c is highly expressed in the liver. Moreover, different studies have shown that SREBPs can directly stimulate the transcription of genes encoding ACC [20–22], FAS enzymes [23], making them good candidates as common regulators of the lipogenetic genes. Therefore, expression of these hepatic lipogenetic genes plays a pivotal role in the process of de novo TG synthesis.

Dehydroepiandrosterone (DHEA, 3β-hydroxy-5-androsten-17-one)-a naturally occurring steroid, is secreted from the adrenal gland as a sulfate ester, which is interconvertible with free DHEA in vivo. DHEA is not a hormone but it is a very important prohormone [24], which exerts various physiological activities through intermediate products when administered to rats and mice. The studies from others have revealed that DHEA has various functions on the regulating of lipid metabolism, which includes decreasing the metabolic efficiency in mammalian species [25, 26], regulating the synthesis of fat, decreasing the number of adipocytes [27–29]. In rodents, long term DHEA treatment resulted in suppression of body weight gain without changes in food intake. During DHEA treatment, liver size is increased with decreasing hepatic lipogenesis [25, 30].

DHEA has a fat-reducing function. However, the mechanism of this physiological role of DHEA has not yet been fully clarified [31, 32]. Moreover, most of previous studies were focused on rodents with only a few studies on chickens and they indicated that DHEA did not significantly suppress body weight of chickens and liver size [33]. No detailed information was available about the effect of DHEA on lipid metabolism in broiler chickens during embryonic development. The fertilized egg would be an appropriate substance in studying the mechanism of DHEA in the regulation of fatty metabolism because the embryo is enclosed in an eggshell, and is hardly influenced by external factors [34].

The objective of the present study was to explore the effect of DHEA on lipid metabolism and hepatic lipogenetic genes expression in broiler chickens during embryonic development which may help to identify the possible mechanism of DHEA in decreasing the deposition of fat in adipose tissue.

Material and Methods

Animal Experiment

Fertilized eggs of laying hens (Arbor Acres) used in this study were obtained from Jiangsu Wuxi Chicken Breeding Company (Wuxi, China). All eggs were numbered and weighed individually prior to the beginning of incubation. Afterwards, eggs were fumigated (80 g potassium permanganate in 130 ml 40% formaldehyde solution per m^3 for 20 min) and randomly divided into two groups. In the control group (CON group), the eggs were injected with 50 μL DMSO, while in DHEA group, the eggs were injected with 50 mg DHEA (Sigma, USA) per kg eggs weight diluted in 50 μL DMSO. All treatments were performed just prior to putting the eggs into the incubator. 50 μL of solution were added to the air sac. Prior to injection, the blunt end of the egg was sterilized with 70% ethanol. A single hole was created with a dental drill bit without penetrating the chorio-allantoic membrane. Each solution was injected into the blunt end of the egg to a depth of 5.0 mm after drilling the shell. Micropipettes were used for injections (Sealpette, Jencons, Finland). After injection, the holes were immediately sealed with melted paraffin wax. They were placed into an electric forced-draft incubator at 37.5 ± 0.5 °C and 60% relative humidity and turned every 2 h. All eggs were incubated in the same incubator. All eggs were candled before incubation and only unchipped and unbroken eggs were used in the experiment. All experimental procedures were performed according to the Guide for Animal Care and Use of Laboratory Animals in the Institutional Animal Care and Use Committee of Nanjing Agricultural University. The experimental protocol was approved by the Departmental Animal Ethics Committee of Nanjing Agricultural University.

Eggs were opened on days 9, 14, 19 of incubation. Samples of liver were collected, weighed and numbered at E9, E14, E19 and at hatching. All liver tissue samples were snap frozen in liquid nitrogen and stored at –80 °C prior to homogenization. Blood samples were collected from the blood vessel at E14d and the heart at E19d with a heparinized syringe. The blood was collected from the jugular vein at hatching. The blood samples were centrifuged at 4 °C, 9,000 × g for 4 min, and the serum was gathered and kept in a –40 °C freezer. The start of incubation was called day 1 (E1d) and after hatching called day 1 (H1).

Measurement of Lipid Parameters

Total liver lipid content was determined on homogenized liver samples using a mixture of chloroform and methanol (2:1 v/v) according to the method of Folch et al. [35]. The levels of hepatic and serum triglyceride (TG) and total cholesterol (TC) content were determined using commercial kits (GPO-PAP and CHOD-PAP) purchased from the Nanjing Jiancheng Bioengineering Institute (NJBI). TG and TC levels in homogenates of liver and serum were evaluated following the manufacturer's protocols. The optic density of the samples was measured using a spectrophotometer three times at a wavelength of 546 nm. Total TG in liver and serum and TC in serum were calculated through absorption of the tested sample divided by standard sample and multiplied by TG and TC content in standard sample.

RNA Extraction

Total RNAs were extracted from the liver using TRIZOL reagent (Takara, Japan) according to the manufacturer's protocols. Total RNA concentration was then quantified by measuring the optic density at 260 nm in a photometer (Eppendorf Biophotometer). Ratios of absorption (260/280 nm) of all preparations were between 1.8 and 2.0. Aliquots of RNA samples were subjected to electrophoresis through a 1.4% agarose formaldehyde gel to verify their integrity.

Real-Time Quantitative RT-PCR

Reverse transcription was performed using the RNA (2 µg) described above in a final volume of 25 µL containing 10 units of MMLV reverse transcriptase (Promega, Belgium), 1 mM dNTP mixture (Promega, Belgium), 40 units of recombinant RNasin ribonuclease inhibitor (Promega, Belgium) and 0.5 µg of oligo (dT) 18 (Promega, Belgium) in sterilized water and buffer supplied by the manufacturer. After incubation at 42 °C for 60 min), the mixture was heat treated at 95 °C for 5 min. An aliquot of cDNA samples was mixed with 25 µl SYBR® Green PCR Master Mix (Takara, Japan), in the presence of 10 pmol of each forward and reverse primer for acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), malic enzyme (ME), apolipoprotein B100 (apoB100) and sterol regulator element binding protein-1c (SREBP-1c) (Table 1), and then subjected to PCR under standard conditions (40 cycles). As an internal control, the same RT products were also subjected to PCR in the presence of a second pair of primers specific to chicken *β*-actin RNA. Mixtures were incubated in an ABI Prism 7300 Sequence Detection System (Applied Biosystems) programmed to conduct one cycle (95 °C for 10 s) and 40 cycles (95 °C for 5 s and 60 °C for 31 s) or one cycle (95 °C for 10 min) and 40 cycles (95 °C for 15 s and 62 °C for 1 min). Results (fold changes) were expressed as 2–ΔΔCt with ΔΔCt = (Ct ij–Ct *β*-actin j)–(Ct i1–Ct *β*-actin1), where Ct ij and Ct*β*-actinj are the Ct for gene i and for *β*-actin in a pool or a sample (named j) and where Ct i1 and Ct *β*-actin1 are the Ct in pool 1 or sample 1, expressed as the standard. All primes used were designed by Primes Premier 5 and synthesized by Shanghai Saibaisheng Biological Company (Shanghai, China).

Statistical Analysis

The results were expressed as mean ± SE and differences were considered significant when $P < 0.05$ tested by two-way analysis of variance (ANOVA) (with treatments and developmental stages as the main effects) and the pair *t*-test with Statistical Packages for Social Science 12.0 and Excel 2003 in Microsoft.

Table 1 Oligonucleotide PCR primers

Gene	Genbank accession number	Primers sequence (5′–3′)	Orientation	Product size (bp)
β-actin	L08165	TGCGTGACATCAAGGAGAAG	Forward	300
		TGCCAGGGTACATTGTGGTA	Reverse	
ACC	J03541	CACTTCGAGGCGAAAAACTC	Forward	447
		GGAGCAAATCCATGACCACT	Reverse	
FAS	J04485	TGAAGGACCTTATCGCATTGC	Forward	195
		GCATGGGAAGCATTTTGTTGT	Reverse	
ME	AF408407	AGCATTACGGTTTAGCATTTCGG	Forward	239
		CAGGTAGGCACTCATAAGGTTTC	Reverse	
SREBP	AY029224	GTCGGCGATCCTGAGGAA	Forward	104
		CTCTTCTGCACGGCCATCTT	Reverse	
ApoB100	M18421	CACGCCTCACACAGACCAAGTA	Forward	407
		CCAGTCAAACGGCACATCTA	Reverse	

Results

Effect of In Ovo Administration of DHEA on Body weights and Liver Weights at Embryonic Stages and Hatching

The effect of in ovo administration of DHEA on body weight, absolute and relative liver weights at embryonic stages and hatching was shown in Table 2. In both groups, body weights sharply increased with developmental stage ($p < 0.05$). Body weight in the CON group was slightly higher than that in the DHEA group throughout the whole embryonic development ($p > 0.05$). Daily gain in the CON group slightly exceeded that of the DHEA group throughout the whole embryonic development ($p > 0.05$).

In both groups, absolute liver weights increased greatly with developmental stage and the values of the control were a little higher than those of in ovo administration of DHEA. But the difference was not significant ($p > 0.05$). In both groups, relative liver weights (g liver/g embryo weight) in the CON group was insignificantly higher than that in the DHEA group during the whole embryonic development ($p > 0.05$).

Effect of In Ovo Administration of DHEA on Plasma and Liver Lipid Metabolism Parameters at Embryonic Stages and Hatching

The effect of in ovo administration of DHEA on total blood TG, TC and hepatic TG content of broiler during embryonic development and at hatching were shown in Table 3. In both groups, the TG content in the liver (mmol/g liver) greatly increased with developmental stages ($p < 0.05$). The TG content in the liver (mmol/g) was similar between CON and DHEA groups at E9d. However, the TG content in CON group was significantly higher than that in DHEA group at E14d, E19d as well as at hatching ($p < 0.05$).

The blood TG (mmol/L) contents in both groups gradually decreased with developmental stages ($p > 0.05$). The TG content in the CON group was significantly higher than that in the DHEA group throughout the whole embryonic development ($p < 0.05$).

The blood TC content remained relatively the same in the two groups during the embryonic development. However, the plasma TC content in the DHEA group was significantly higher than that in the CON group at hatching ($p < 0.05$).

Table 2 Effect of in ovo administration of DHEA on body weight, liver weight and lipid parameters during embryonic development

	E9	E14	E19	H1
Body weight (g)				
CON	2.12 ± 0.25^{a}	12.55 ± 1.59^{b}	29.36 ± 3.25^{c}	40.69 ± 2.97^{c}
DHEA	2.01 ± 0.23^{A}	12.46 ± 1.54^{B}	29.27 ± 4.35^{C}	39.31 ± 3.15^{C}
Absolute liver weight (g)				
CON	0.04 ± 0.01^{a}	0.22 ± 0.04^{b}	0.52 ± 0.11^{c}	0.87 ± 0.12^{d}
DHEA	0.04 ± 0.01^{A}	0.20 ± 0.05^{B}	0.50 ± 0.10^{C}	0.80 ± 0.11^{D}
Relative liver weight (%)				
CON	1.78 ± 0.23^{a}	1.80 ± 0.21^{a}	1.77 ± 0.27^{a}	2.19 ± 0.31^{a}
DHEA	1.75 ± 0.27^{A}	1.56 ± 0.28^{A}	1.70 ± 0.28^{A}	1.99 ± 0.31^{A}
Daily gain (g/d)				
CON		2.09 ± 0.27^{a}	3.36 ± 0.76^{b}	2.28 ± 0.23^{b}
DHEA		2.08 ± 0.25^{A}	3.32 ± 0.71^{B}	2.11 ± 0.31^{B}

Means ± SE without a common letter differ significantly between age groups (small letter for CON group and capital letter for DHEA treatment).
* Treatment differences at the same age ($P < 0.05$) ($n = 10$)

Table 3 Effect of in ovo administration of DHEA on lipid parameters during embryonic development

	E9	E14	E19	H1
Hepatic TG (mmol/g liver)				
CON	4.45 ± 0.51^{a}	$19.43 \pm 1.55^{b*}$	$22.25 \pm 1.52^{b*}$	$25.54 \pm 1.21^{b*}$
DHEA	4.01 ± 0.43^{A}	12.62 ± 1.22^{B}	19.33 ± 1.43^{B}	21.74 ± 1.44^{B}
Plasma TG (mmol/L)				
CON		$5.11 \pm 0.55^{a*}$	2.93 ± 0.35^{b}	$2.25 \pm 0.55^{c*}$
DHEA		3.60 ± 0.41^{A}	2.31 ± 0.28^{B}	1.40 ± 0.43^{C}
Plasma TC (mmol/L)				
CON		3.05 ± 1.36^{b}	7.04 ± 2.74^{c}	$7.35 \pm 2.07^{c*}$
DHEA		4.58 ± 2.17^{B}	6.45 ± 3.66^{C}	9.96 ± 2.89^{C}

Means ± SE without common letter differ significantly between age groups (small letter for CON group and capital letter for DHEA treatment).
* Treatment differences at the same age ($P < 0.05$) ($n = 10$)

Effect of In Ovo Administration of DHEA on Hepatic ACC Gene Expression During Embryonic Development and at Hatching

The effect of in ovo administration of DHEA on ACC gene expression during embryonic development and at hatching was investigated in this study and the results were shown in Fig 1. mRNA level of *ACC* increased with embryonic development proceeding, but it decreased significantly at hatching in both groups ($p < 0.05$). Expression level of the *ACC* gene in CON group was significantly lower than that in DHEA group during embryonic 19 days ($p < 0.05$). However, *ACC* gene expression in CON group was four fold higher than that in DHEA group at hatching.

Effect of In Ovo Administration of DHEA on Hepatic *FAS* Gene Expression During Embryonic Development and at Hatching

We then studied the effect of in ovo administration of DHEA on *FAS* gene expression during embryonic development. The result showed that *FAS* gene expression levels were nearly the same in both groups during embryonic development (Fig 2). *FAS* gene expression of CON was 18-fold higher than that in DHEA group at hatching and this difference was significant ($p < 0.05$). *FAS* gene expression exhibited an increase in the CON group during embryonic development and at hatching. However, FAS gene expression decreased sharply in DHEA group at hatching ($p < 0.05$).

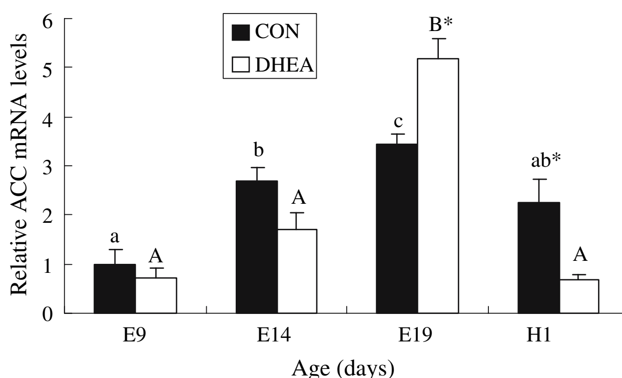

Fig. 1 Effect of in ovo administration of DHEA on hepatic *ACC* gene expression during embryonic development and at hatching. RNA molecules extracted from liver of different stages of embryonic development or hatching were reverse transcribed to cDNA and analyzed by quantitative RT-PCR. For comparison between different samples, the *ACC* transcript level of each sample was normalized for the beta-actin level and expressed as a multiple of *ACC* level of E9. Each column represented the mean and standard error of results obtained with 6 experiments. *Black bars*, control group (CON); *Blank bars*, DHEA group (DHEA); *Asterisks* indicate the differences between CON and DHEA group are significant ($P < 0.05$)

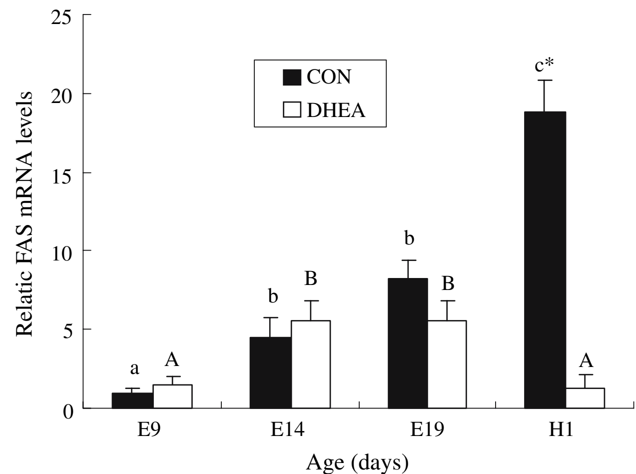

Fig. 2 The effect of in ovo administration of DHEA on hepatic *FAS* gene expression during embryonic development and at hatching–RNA molecules were extracted from the liver atdifferent stages of embryonic development or at hatching and were reverse transcribed to cDNA and analyzed by quantitative RT-PCR. For comparison between different samples, the *FAS* transcript level of each sample was normalized for the beta-actin level and expressed as a multiple of the *FAS* level of E9. Each column represented the mean and standard error of results obtained with six experiments. *Black bars*, control group (CON); *Blank bars*, DHEA group (DHEA); *Asterisks* indicate the differences between CON and DHEA group are significant ($P < 0.05$)

Effect of In Ovo Administration of DHEA on Hepatic SREBP-1c Gene Expression During Embryonic Development and at Hatching

The result of in ovo administration of DHEA on hepatic *SREBP-1c* gene expression during embryonic development and at hatching was shown in Fig. 3. *SREBP-1c* gene expression levels were the same in the DHEA group as in the CON group during the embryonic development. At hatching, *SREBP-1c* transcript level in the CON group was three-fold higher than that in the DHEA group. The difference was significant ($p < 0.05$). *SREBP-1c* gene expression exhibited increasing with embryonic development in two groups. However, the expression level of *SREBP-1c* in the CON group increased more than that in the DHEA group.

The Effect of in Ovo Administration of DHEA on Hepatic *ME* Gene Expression During Embryonic Development and at Hatching

In this study, we also investigate the effect of in ovo administration of DHEA on *ME* gene expression during embryonic development and at hatching. The result was shown in Fig. 4. The transcript level of *ME* in CON group was significantly higher than that in DHEA group from embryonic development of 14 days to hatching ($p < 0.05$).

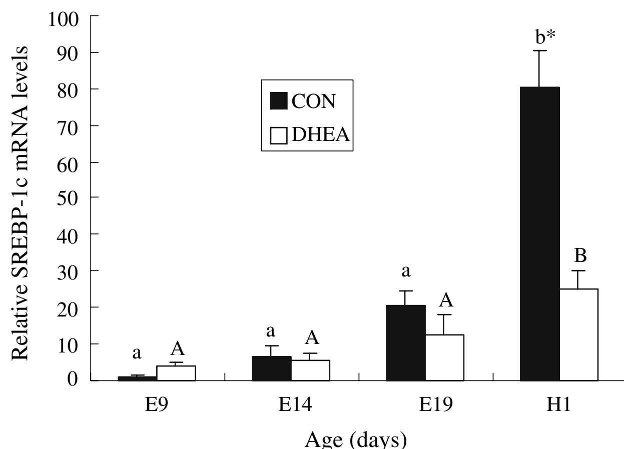

Fig. 3 The effect of in ovo administration of DHEA on hepatic *SREBP-1c* gene expression during embryonic development and at hatching. RNA molecules extracted from liver of different stages of embryonic development or hatching were reverse transcribed to cDNA and analyzed by quantitative RT-PCR. For comparison between different samples, the *SREBP-1c* transcript level of each sample was normalized for the beta-actin level and expressed as a multiple of *SREBP-1c* level of E9. Each column represented the mean and standard error of results obtained with six experiments. *Black bars*, control group (CON); *Blank bars*, DHEA group (DHEA); *Asterisks* indicate that the differences between CON and DHEA groups are significant ($P < 0.05$)

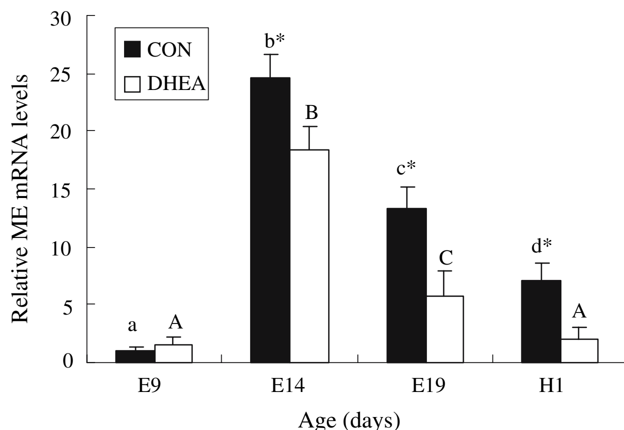

Fig. 4 The effect of in ovo administration of DHEA on hepatic *ME* gene expression during embryonic development and at hatching. RNA molecules extracted from liver of different stages of embryonic development or hatching were reverse transcribed to cDNA and analyzed by quantitative RT-PCR. For comparison between different samples, the *ME* transcript level of each sample was normalized for the beta-actin level and expressed as a multiple of *ME* level of E9. Each column represented the mean and standard error of results obtained with 6 experiments. *Black bars*, control group (CON); *Blank bars*, DHEA group (DHEA); *Asterisks* indicate the differences between CON and DHEA group are significant ($P < 0.05$)

Moreover, the CON to DHEA ratio of expression level increased with embryonic development. The expression level of *ME* decreased from E14 days to hatching.

Effect of In Ovo Administration of DHEA on Hepatic *ApoB* Gene Expression During Embryonic Development and at Hatching

Finally, we studied the effect of in ovo administration of DHEA on *apoB* gene expression during embryonic development and at hatching, and the result was shown in Fig. 5. The expression level of the hepatic *apoB* gene significantly increased through the course of embryonic development ($p < 0.05$). Measured by RT-PCR, the mRNA level of *apoB* in the CON group was significantly higher than that in DHEA treatment throughout all the stages of embryonic development ($p < 0.05$).

Discussion

Long term DHEA treatment results in suppression of body weight gain in rodents [36, 37]. However, no significant effect of in ovo administration of DHEA on body weight, body weight gain and absolute liver weight was observed in this experiment. In contrast to its effects on rats and mice, DHEA did not significantly depress body weight of chickens [33]. Rats fed with DHEA had a slower growth rate compared with rats fed with a control diet, whereas rats treated with DHEA i.p. had growth rates identical to those of controls. The liver weights of rats administrated DHEA p.o. or i.p. increased significantly compared to those of control rats [39]. In lean rats, DHEA treatment did not decrease liver weight [38]. Liver weights of DHEA treated rats were higher than those of control rats [39]. Administration of DHEA to rats results in lowered body weight, higher liver weights and DNA, RNA, and/or protein content, but lowered lipid and glycogen levels [40]. These data indicate that the effect of DHEA on body weight gain, food intake, and hepatic and peripheral adiposity are dependent on the species of animal, the adrenal status, the DHEA dose and the age of embryonic development at which measurements were made [41, 42].

DHEA has a fat-reducing effect, however, this effect may be exerted by different mechanisms [31]. It has been shown in this study that in ovo administration of DHEA to fertilized eggs before incubation reduced plasma and liver TG content. This result agreed with other studies in rodents. For example, the study by Mikheil and Leila [43] indicated that dehydroepiandrosterone influences on lipid metabolism - reduces the levels of TC, TG, LDL, VLDL. Mohan. PF and Cleary. MP [39, 40] suggested DHEA treatment reduced hepatic lipids in rats and under some circumstances, altered a number of serum factors including glucose, insulin, cholesterol, and triacylglycerol. Therefore, DHEA may reduce the adipose tissue by reducing the TG content in adipose tissue.

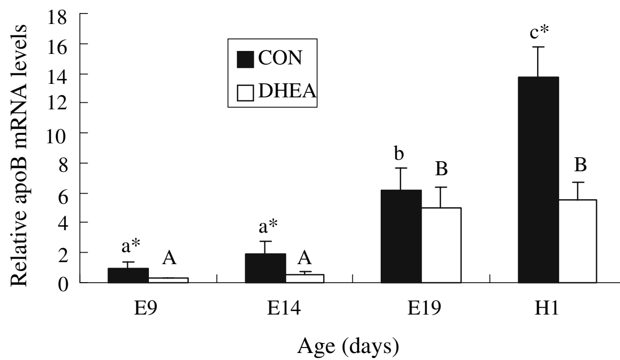

Fig. 5 The effect of in ovo administration of DHEA on hepatic *apoB* gene expression during embryonic development and at hatching. RNA molecules extracted from liver of different stages of embryonic development or hatching were reverse transcripted to cDNA and analyzed by quantitative RT-PCR. For comparison between different samples, the *apoB* transcript level of each sample was normalized for the beta-actin level and expressed as a multiple of the *apoB* level of E9. Each column represented the mean and standard error of results obtained with six experiments. *Black bars*, control group (CON); *Blank bars*, DHEA group (DHEA); *Asterisks* indicate the differences between CON and DHEA group are significant ($P < 0.05$)

Other studies also indicated that DHEA treatment altered the activity levels of a lot of enzymes in the liver that are involved in lipid metabolism [40]. This could be one of the mechanisms of DHEA for decreasing the fat level of the animal. For example, Valentine et al. [33] reported that liver cytosolic malic enzyme activity were depressed in chickens treated with DHEA compared with that in untreated animals. This study showed that the expression level of the hepatic *ME* gene in DHEA group was lower than that in the CON group. The relative abundances of malic enzyme mRNAs were associated with malic enzyme activity and liver total lipid concentration [44]. The activity of the hepatic malic enzyme is positively correlated with the rate of fatty acid synthesis, the percentage of body fat, and the percentage of abdominal fat in chicks. Meanwhile, Casazza et al. [45] hypothesized that DHEA inhibited fat synthesis by diminishing the availability of NADPH, but no data gave support to the hypothesis that administration of DHEA resulted in decreased cytoplasmic NADPH in the liver of rats. In the avian liver, most of the NADPH used by fatty acid synthase to catalyze the synthesis of palmitate is generated by the malic enzyme [14]. Therefore, DHEA reduced hepatic *ME* gene expression so as to decrease the hepatic fatty acid synthesis and de novo TG synthesis.

In our published study, we suggested that expression of the key gene (*FAS*) in the liver was responsible for de novo synthesis of fatty acid prior to hatching and at hatching [46]. The rate of hepatic lipogenesis was decreased by approximately 70% in DHEA-treated mice [47]. Noteworthy, the fat-reducing effect of DHEA seems to be more evident at the level of visceral adipose tissue [31, 41]. Moreover, this study showed that DHEA significantly depressed the *FAS* gene expression at hatching. This means that DHEA decreased FAS gene expression to reducing fat especially at hatching.

In avian species, the development of adipose tissue depends on the availability of plasma triglycerides that are hydrolyzed prior to their uptake by adipocytes. They are specifically transported to adipocytes by specific lipoprotein classes: very low density lipoproteins (VLDL) transport de novo synthesized hepatic lipids [4, 5]. *ApoB* participated in the assembly of VLDL. This study showed DHEA decreased the *apoB* gene expression, which suggested that DHEA depressed the assembly of VLDL, resulting in the suppression of the transportation of hepatic TG to various tissues.

As one of potential regulators, *SREBP-1c* can directly stimulate the transcription of genes encoding *ACC* [20–22], *FAS* [23] enzymes. In this study, DHEA decreased the expression of both *SREBP-1c* and *FAS* genes throughout the whole embryonic development. However, DHEA significantly reduced the expression of all three genes, *SREBP-1c*, *ACC* and *FAS* at hatching. Our previous studies indicated that embryonic liver synthesized fatty acid prior to hatching or at hatching [46]. This suggested that by decreasing the expression of transcriptional regulator, DHEA altered the expression levels of various downstream genes that are involved in the synthesis of TG.

In conclusion, this study analyzed the effect of in ovo administration of DHEA on blood lipid metabolism and hepatic lipogenetic genes expression in broiler chicken during embryonic development. The result indicated that DHEA significantly decreased liver weight, blood TG content, hepatic TG content and the expression levels of hepatic *ACC*, *FAS*, *ME*, *SREBP-1c* and *apoB* genes in broiler chicken during embryonic development. All of these affected genes are involved in the metabolism of fat tissue. A higher rate of triglyceride synthesis and secretion from the liver would be responsible for the higher abdominal fat weight in chickens. Over all, our data suggested that DHEA decreased the synthesis of TG in liver and its transport in circulation, which is probably the mechanism of DHEA in reducing the accumulation of fat in chicken.

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