Gluconeogenesis is the process by which glucose is generated from noncarbohydrate sources such as amino acids, propionate, and glycerol. In vertebrates, gluconeogenesis occurs mainly in the liver and, to a lesser extent, in the kidneys. Hepatic gluconeogenesis plays a key role in chicken embryonic development, and it plays a major role in glucose homeostasis for developing embryos. Phosphoenolpyruvate carboxylase (PEPCK) catalyzes the rate-limiting step of gluconeogenesis, yet how hepatic PEPCK expression is differentially regulated between chicken breeds remains elusive. In this study, fertile eggs from a slow-growing Chinese Yellow Feathered Chicken and a fast-growing White Recessive Rock Chicken were incubated under the same standard conditions, and serum and liver samples were collected on embryonic d 18 (18E). The fast-growing breed had a significantly higher fetal weight \((P < 0.01)\) and serum glucose concentration \((P < 0.05)\) compared with the slow-growing breed. The fast-growing breed also had significantly higher hepatic mRNA expression levels of the cystolic form of PEPCK (PEPCK-c; \(P < 0.05\)) and significantly higher hepatic mRNA and protein expression levels of cAMP response element binding protein 1 (CREB-1; \(P < 0.05\)). Moreover, the binding of phosphorylated CREB-1 to the PEPCK-c promoter tended to be higher in the fast-growing breed \((P = 0.08)\). Breed-specific epigenetic modifications of the PEPCK-c promoter were also observed; the fast-growing breed demonstrated lower CpG methylation \((P < 0.05)\) and histone H3 \((P < 0.05)\) levels but more histone H3 acetylation \((H3\text{ac})\) and histone H3 lysine 27 trimethylation \((H3\text{K27me3}; P < 0.05)\) compared with the slow-growing breed. Our results suggest that hepatic PEPCK-c expression is transcriptionally regulated in a breed-specific manner and that fast- and slow-growing broiler chicken fetuses exhibit different epigenetic modifications on their PEPCK-c promoter regions.

**Key words:** broiler, liver, phosphoenolpyruvate carboxylase, transcription regulation, gluconeogenesis

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**INTRODUCTION**

Gluconeogenesis is the process by which glucose is generated from noncarbohydrate sources such as amino acids, propionate, and glycerol. In vertebrates, gluconeogenesis occurs mainly in the liver and, to a lesser extent, in the kidneys. Hepatic gluconeogenesis plays a key role in chicken embryo development, especially during the final week, when the fetus prepares for hatching (De Oliveira et al., 2008). Muscle grows rapidly and glycogen stores increase dramatically starting at internal pipping and continuing to hatching. During this time, hepatic gluconeogenesis becomes the sole source of glucose and glycogen (Moran, 2007; De Oliveira et al., 2008; Pulikanti et al., 2010). At this stage, deregulation of hepatic gluconeogenesis or abnormal glycogen storage can either cause a failure to hatch or affect chick vitality after hatching.

Different chicken breeds show striking differences in growth and metabolism. However, comparative studies on hepatic gluconeogenesis in different chicken breeds are scarce. Serum glucose concentration is higher in broilers than in layers (Mahagna and Nir, 1996), but the hepatic glycogen content among chicken breeds does not differ (O’Dea et al., 2004). In the final stage of embryo development, the substrate for hepatic gluconeogenesis is mainly the amino acids derived from protein digestion in amniotic fluid and tissue storage (Moran, 2007). It has been shown that the amino acid contents in breeder eggs are different between fast- and slow-growing broiler breeds (Zeng et al., 2011); however, it remains to be determined if hepatic gluconeogenesis differs between breeds during the final period of embryonic development.

Phosphoenolpyruvate carboxylase (PEPCK) is a key enzyme of gluconeogenesis, which catalyzes the rate-limiting step of phosphoenolpyruvate formation by
decarboxylating oxaloacetate (Beale et al., 1985). Phosphoenolpyruvate carboxylase has 2 isoforms, a cytosolic form (PEPCK-c) and a mitochondrial form (PEPCK-m), which are encoded by 2 different genes (Weldon et al., 1990). The PEPCK-c gene is activated at birth and remains active in the liver of mammals throughout life. In birds, however, PEPCK-c is abundantly expressed in the liver during the embryonic period, and this expression decreases to negligible levels after hatching (Savon et al., 1997). Expression of PEPCK-m is stable and almost unresponsive to hormonal influences, whereas PEPCK-c expression is highly sensitive to nutrition, stress, and endocrine status (Watford et al., 1981). It has been shown that the influences of glucagon, glucocorticoids, and insulin on PEPCK-c expression are regulated at the transcriptional level (Watford et al., 1981). Using 2-dimensional electrophoresis, Huang et al. (2010) found a difference between breeds in the hepatic content of PEPCK protein on embryonic d 19. However, breed-specific differences in hepatic PEPCK-c transcriptional regulation in chick embryos have not been reported so far.

Several transcription factors have been reported to be involved in the transcriptional regulation of PEPCK-c, including the cAMP response element binding protein 1 (CREB1). The CREB1 is phosphorylated by several protein kinases, and it induces gene transcription in response to hormonal stimulation of the cAMP pathway by binding to the cAMP response element (CRE) located on gene promoters (Scott et al., 1998; Leahy et al., 1999). The DNase I footprint analysis revealed that CREB1 binds to the CRE located on the avian PEPCK-c promoter immediately 5′ of the transcription start-site (Savon et al., 1997). However, it remains unknown whether there are breed-specific differences in CREB1 expression and CREB1 binding of the PEPCK-c gene promoter.

Epigenetic mechanisms, including DNA methylation and histone modifications, play important roles in the transcriptional regulation of gene expression. Maternal protein restriction induces changes in the PEPCK-c promoter methylation patterns in the liver of offspring rats (Burdge et al., 2007). Fast- and slow-growing broiler breeds are fed diets with different protein levels; therefore, it is intriguing to determine whether epigenetic modifications on the PEPCK-c promoter in liver cells differ between breeds and how the breed-dependent epigenetic status is associated with hepatic PEPCK-c expression in chicken fetuses.

The present study aimed to compare the hepatic expression of PEPCK-c between the Chinese Yellow Feathered Chicken (slow-growing) and the White Recessive Rock Chicken (fast-growing) on embryonic d (E) 18 (18E) and to explore the involvement of CREB1 and epigenetic modifications in the breed-specific transcriptional regulation of PEPCK-c in the liver of chicken fetuses during the final stage of embryonic development.

MATERIALS AND METHODS

Birds and Experimental Design

Fertile eggs laid by the slow-growing Chinese Yellow Feathered Chicken and the fast-growing White Recessive Rock Chicken were obtained from Southern Poultry Breeding Company of WENS Co. Ltd., Guangdong, China. The eggs were incubated in an electric forced-draft incubator at 37.5 ± 0.5°C and 60% RH. The first day of incubation was defined as 1E. On 18E, fetuses were killed, and blood and liver samples were collected. Serum was prepared and kept in a −70°C freezer. All liver samples were snap frozen in liquid nitrogen and stored at −70°C before homogenization. All experimental procedures were approved by the Animal Ethics Committee of Nanjing Agricultural University.

Serum Glucose and Hepatic Glycogen Analysis

Serum glucose concentrations were measured in duplicate using a commercial kit (Nanjing Jiancheng, Nanjing, China) according to the manufacturer’s guidelines. Hepatic glycogen was extracted and analyzed according to a previous publication (Bennett et al., 2007).

Quantitation of mRNA by Real-Time PCR

Total RNA was extracted from livers with TRIzol reagent (Invitrogen, Carlsbad, CA) and treated with DNase I (D2215, Takara Bio, Otsu, Japan) to prevent possible contamination of genomic DNA. One microgram of total RNA was reverse transcribed, and real-time PCR was performed in Mx3000P (Stratagene, La Jolla, CA). Two microliters of 20-fold dilution of RT product was used for PCR in a final volume of 25 µL containing 12.5 µL of SYBR Green Real-time PCR Master Mix (TaKaRa Bio). All the primers are listed in Table 1, and chicken β-actin was selected as a reference gene. The following PCR protocols were initial denaturation (3 min at 95°C), then a 3-step amplification program (20 s at 95°C, 20 s at 62°C, 20 s at 72°C) repeated 40 times. The 2−ΔΔCt method was used to analyze the real-time PCR data (Livak and Schmittgen, 2001), and the results presented as the fold change relative to the average value of the slow-growing breed.

Western Blotting

Protein extracts from frozen liver samples were prepared as previously described [Yuan et al., 2009]. Protein extracts (20 µg) were subjected to electrophoresis on a 10% SDS-PAGE gel, and the separated proteins were transferred onto nitrocellulose membranes (Pall Co., New York, NY). Western blot analysis for PEPCK-c (Proteintech Group, Chicago, IL; 1:1,000) and pCREB1 (Santa Cruz, Dallas, TX; 1:1,000) was
performed with their respective primary antibodies and corresponding horseradish peroxidase-conjugated secondary antibodies; GAPDH (AP0066, Bioworld, Minneapolis, MN; 1:10,000) was used as a reference. Finally, the blots were detected by enhanced chemiluminescence

Table 1. Primer sequences used in real-time PCR analysis

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Product length (bp)</th>
<th>Sequence$^2$</th>
<th>GenBank accession no.</th>
<th>Applications$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEPCK-c</td>
<td>284</td>
<td>F: 5′-GAGACCTGTCGCCCTCACCA-3′</td>
<td>NM_205471</td>
<td>mRNA quantification</td>
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<tr>
<td>PEPCK-m</td>
<td>294</td>
<td>R: 5′-CGACCCAGCTGCTTACCTT-3′</td>
<td>NM_204570</td>
<td></td>
</tr>
<tr>
<td>FBP1</td>
<td>102</td>
<td>F: 5′-TGCTGTCAGGAGATGTG-3′</td>
<td>AJ276122</td>
<td></td>
</tr>
<tr>
<td>GR</td>
<td>102</td>
<td>R: 5′-CTCCATCCGCCTCCTTCA-3′</td>
<td>DQ227738</td>
<td></td>
</tr>
<tr>
<td>CREB1</td>
<td>187</td>
<td>F: 5′-CAGACGACCCTCCTTACA-3′</td>
<td>NM_204450</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>300</td>
<td>R: 5′-AATTGCTTCTCTCTGTGC-3′</td>
<td>L08165</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>105</td>
<td>F: 5′-CAGGTCACAGACGCTG-3′</td>
<td>ENSGALT00000012356</td>
<td>MeDIP and ChIP</td>
</tr>
<tr>
<td>P2</td>
<td>200</td>
<td>R: 5′-CCAACTGGCAGACATC-3′</td>
<td>ENSGALT00000012356</td>
<td>ChIP</td>
</tr>
<tr>
<td>pCREB1</td>
<td>120</td>
<td>R: 5′-AAAGGTTTCCCTTGTGCATCC-3′</td>
<td>ENSGALT00000012356</td>
<td></td>
</tr>
<tr>
<td>ACTB</td>
<td>121</td>
<td>R: 5′-TCCTGCTTCCCCGTTGTGC-3′</td>
<td>ENSGALT0000039969</td>
<td>MeDIP</td>
</tr>
</tbody>
</table>

$^1$PEPCK-c = phosphoenolpyruvate carboxylase, cytoplasmic form; PEPCK-m = phosphoenolpyruvate carboxylase, mitochondrial form; FBP1 = fructose-1, 6-bisphosphatase; GR = glucocorticoid receptor; CREB1 = cAMP response element binding protein 1; P1 = primer 1; P2 = primer 2; pCREB1 = phosphorylated CREB1; ACTB = β-actin

$^2$F: forward, R: reverse.

$^3$MeDIP = methylated DNA immunoprecipitation; ChIP = chromatin immunoprecipitation.

The enhanced chemiluminescence signals were recorded using the LumiGlo substrate (Pierce, Rockford, IL). The enhanced chemiluminescence signals were recorded by an imaging system (Bio-Rad, Philadelphia, PA) and analyzed with Quantity One software (Bio-Rad).

**Chromatin Immunoprecipitation Assay**

Chromatin immunoprecipitation (ChIP) analysis was performed according to our previous publication (Jia et al., 2012). Briefly, liver samples were ground in liquid nitrogen and washed with PBS containing a protease inhibitor cocktail (Roche, Basel, Switzerland). After crosslinking, samples were sonicated to produce 300- to 500-bp DNA fragments. The crude chromatin preparations were precleared with 40 µL of protein A/G agarose beads and then incubated with their respective primary antibodies (Table 2). A negative control was included using normal rat IgG. Immune complexes were captured with the beads, and DNA fragments were released by reverse cross-linking. Purified ChIP DNA was used to amplify the PEPCK-c gene promoter sequences by real-time PCR with specific primers (Table 1). The specificity of each ChIP sample was validated by determining the differences in the Ct values ($ΔCt$ > 6) of DNA templates precipitated by a specific antibody compared with those precipitated with normal rat IgG. The ChIP results were calculated for each input and were presented as the exponential degree of change relative to the average MeDIP value of the slow-growing breed.

**Statistical Analysis**

All statistical analyses were performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). All data were expressed as the mean ± SEM. Comparisons between groups were performed using t-tests for indepen-
dent samples. The level of significance was set at $P < 0.05$ for all analyses.

RESULTS

BW, Relative Liver Weight, Serum Glucose Level, and Hepatic Glycogen Content

Egg weight and fetal weight on 18E were significantly ($P < 0.05$) higher in the fast-growing breed compared with the slow-growing one (Table 3). Although liver weight was not significantly different between the 2 breeds, the ratio of liver weight to fetus weight (liver index) on 18E was significantly ($P < 0.05$) lower in the fast-growing breed. Moreover, the serum glucose level was significantly higher ($P < 0.05$) in the fast-growing breed, although the hepatic glycogen content did not differ between breeds (Table 3).

Hepatic Expression of PEPCK-c and CREB1

The fast-growing breed showed significantly higher ($P < 0.05$) hepatic PEPCK-c mRNA expression (Figure 1a) compared with the slow-growing breed, yet PEPCK-m did not show a breed difference (Figure 1b). No breed difference was observed for the hepatic content of PEPCK-c protein (Figure 1c and 1d). However, hepatic CREB1 mRNA (Figure 2a) and phosphorylated CREB1 protein (Figure 2b and 2c) were significantly higher ($P < 0.05$) in fast-growing breed.

pCREB1 Binding and Epigenetic Modifications on PEPCK-c Promoter

Two CpG islands ($−2,740$ to $−2,488$, $−577$ to $−339$) and one CRE ($−85$ to $−78$) are predicted to be in the chicken PEPCK-c gene promoter (Figure 3a). No sequence differences were found in these 3 promoter regions between the 2 breeds. Two pairs of primers (P1 and P2) flanking the 2 CpG islands were used to amplify the respective fragments of chicken PEPCK-c gene promoter from MeDIP DNA. The methylation levels of only the first CpG island were significantly lower ($P < 0.05$) in the fast-growing breed compared with the slow-growing one (Figure 3b). Moreover, pCREB1 binding to the CRE in the PEPCK-c gene promoter tended to be higher ($P = 0.08$) in the liver of fast-growing broiler fetuses (Figure 3c). The ChIP analysis also demonstrated breed-specific differences in the histone modifications surrounding the PEPCK-c promoter. Fast-growing broiler fetuses demonstrated lower histone H3 ($P < 0.05$) levels, but they had increased histone H3 acetylation (H3ac; $P < 0.05$) and histone H3 lysine 27 trimethylation (H3K27me3; $P < 0.05$) on the proximal region of the PEPCK-c gene promoter (Figure 3d and 3e), associated with higher hepatic PEPCK-c mRNA expression.

DISCUSSION

To our knowledge, this report is the first on the breed-specific regulation of hepatic PEPCK-c gene transcription in chicken fetuses. As a rate-limiting enzyme of gluconeogenesis, PEPCK has received considerable attention in medical studies. Mouse studies on PEPCK gene knockouts and overexpression have linked the 2 genes encoding the 2 PEPCK isozymes, PEPCK-c and PEPCK-m, to diabetes and obesity (Beale et al., 2007). Expression of PEPCK is usually positively correlated with blood glucose level in alloxan-induced (Yang et al., 2012) and genetic db/db (Huang et al., 2012) diabetic mouse models. Compared with mammals, chickens are naturally hyperglycemic and insulin resistant (Ji et al., 2012). Fast-growing broilers are reported to have higher serum glucose concentrations than slow-growing layer chickens (Mahagna and Nir, 1996), which is in line with our finding that fast-growing broiler fetuses had higher serum glucose levels than the slow-growing ones. In

Table 2. Chromatin immunoprecipitation antibodies

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Species</th>
<th>Source</th>
<th>Catalogue no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>Rabbit</td>
<td>Millipore</td>
<td>12–370</td>
</tr>
<tr>
<td>H3</td>
<td>Rabbit</td>
<td>Abcam</td>
<td>ab1791</td>
</tr>
<tr>
<td>H3ac</td>
<td>Rabbit</td>
<td>Millipore</td>
<td>17–615</td>
</tr>
<tr>
<td>H3K27me3</td>
<td>Rabbit</td>
<td>Millipore</td>
<td>17–622</td>
</tr>
<tr>
<td>pCREB1</td>
<td>Rabbit</td>
<td>Santa Cruz</td>
<td>sc-7978-R</td>
</tr>
</tbody>
</table>

Table 3. Egg weight, fetus weight, and liver weight of broiler chicken fetuses on embryonic d 18

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Slow</th>
<th>Fast</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg weight (g, n = 40)</td>
<td>45.13 ± 0.17</td>
<td>54.79 ± 0.16</td>
<td>0.00</td>
</tr>
<tr>
<td>Fetus weight (g, n = 40)</td>
<td>21.03 ± 0.23</td>
<td>25.41 ± 0.21</td>
<td>0.00</td>
</tr>
<tr>
<td>Liver weight (mg, n = 40)</td>
<td>503.05 ± 8.72</td>
<td>519.11 ± 6.87</td>
<td>0.15</td>
</tr>
<tr>
<td>Liver index (%) (n = 40)</td>
<td>2.39 ± 0.03</td>
<td>2.05 ± 0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Glycogen (mg/g of liver, n = 5)</td>
<td>2.52 ± 1.69</td>
<td>8.73 ± 0.36</td>
<td>0.03</td>
</tr>
</tbody>
</table>

1Values are mean ± SEM. Slow, slow-growing breed; fast, fast-growing breed.

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general, blood glucose concentration is determined by several factors including intestinal absorption, hepatic production, tissue uptake, and renal reabsorption. During the final period of chicken embryonic development, oxygen supply is limited, and hepatic gluconeogenesis becomes the only source of glucose (De Oliveira, 2008). In fact, fructose-1, 6-bisphosphatase (FBP1), another key enzyme of gluconeogenesis, was also expressed at significantly higher ($P < 0.05$) levels in the liver of fast-growing chicken fetuses (data not shown). Therefore, it appears that fast-growing chicken fetuses may have enhanced hepatic gluconeogenesis compared with their higher serum glucose concentration. However, hepatic content of the PEPCK-c protein was not different between the 2 breeds. The uncoupling of PEPCK-c mRNA and protein could be attributed to the differences in assay sensitivity, high individual variation, and limited sample numbers. It is also possible that PEPCK mRNA is translated less efficiently in fast-growing than in slow-growing chicken embryos. Moreover, the differential gluconeogenic activity between the 2 breeds may also be mediated by differences in the expression or activity (or both) of other enzymes involved in gluconeogenesis. In contrast to PEPCK-c, PEPCK-m mRNA expression was not dependent on breed, which is in

![Figure 1](image1.png)

**Figure 1.** Expression of liver PEPCK-c (a) and PEPCK-m (b) mRNA as well as PEPCK-c protein (c and d) in slow- (■) and fast- (□) growing broiler chicken fetuses. Values are means ± SEM, $n = 5$/group. *$P < 0.05$ vs. slow-growing breed. PEPCK-c, cytosolic form of phosphoenolpyruvate carboxykinase; PEPCK-m, mitochondrial form of phosphoenolpyruvate carboxykinase; fast, fast-growing breed; slow, slow-growing breed; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

![Figure 2](image2.png)

**Figure 2.** Expression of liver CREB1 mRNA (a) and protein (b, c, and d) in slow- (■) and fast- (□) growing broiler chicken fetuses. Values are means ± SEM, $n = 3$ to 5/group. *$P < 0.05$ vs. slow-growing breed. CREB1, cAMP response element binding protein; fast, fast-growing breed; slow, slow-growing breed; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
accordance with a previous report, which stated that hepatic PEPCK-m was not significantly affected by fasting in quails (Sartori et al., 2000).

In mammals, several transcription factors have been reported to participate in the transcriptional regulation of the PEPCK-c gene, including CCAAT/enhancer-binding protein β (C/EBP β; Eubank et al., 2001), activating transcription factor 2 (ATF2; Lee et al., 2002), and CREB1 (Savon et al., 1997). No breed-specific difference was detected in this study for C/EBP β and ATF2 (data not shown); however, the hepatic expression of total CREB1 mRNA and pCREB1 protein was found to be significantly higher in the fast-growing breed. This result implicates the involvement of CREB1 in the transcriptional regulation of the PEPCK-c gene in chicken fetuses.

Using DNase I footprint analysis, chicken CREB1 was previously found to bind to the CRE on the avian PEPCK-c promoter (Savon et al., 1997). By using ChIP analysis, we provide further evidence that phosphorylated CREB1 can bind to the CRE of the chicken PEPCK-c promoter in the liver and that the binding tends to be more saturated in fast-growing chicken fetuses. The tendency of enhanced pCREB1 binding to the PEPCK-c promoter is associated with higher PEPCK-c gene expression, indicating that pCREB1 positively regulates PEPCK-c transcription in the liver of chicken fetuses.
Epigenetics plays an important role in the transcriptional regulation of functional genes. The PEPCK mRNA expression is inversely related to the methylation of specific CpG dinucleotides on its promoter in rats (Burdge et al., 2011). Accordingly, we also detected a difference in the DNA methylation levels of the first CpG island in the PEPCK-c promoter between the livers of fast- and slow-growing chicken fetuses. Nevertheless, we could not conclude, from this result, which specific CpG sites are involved in transcriptionally regulating the PEPCK-c gene in the liver of chicken fetuses. The MeDIP analysis employed in this study could only provide the overall methylation status of DNA fragments within the PEPCK-c gene promoter. To quantify the changes in the methylation levels for each individual CpG site, bisulfite sequencing or Epi-TYPER-MassARRAY assay (Sequenom Inc.) must be used to achieve higher resolution.

Histone modifications are also involved in the regulation of gene transcription, and generally, the patterns of DNA methylation and histone modifications on a specific locus are closely associated (Lindroth et al., 2008). However, although a breed-specific difference in DNA methylation was detected in this study only in the first CpG island of the PEPCK-c promoter, we were able to detect differences in histone modifications on the PEPCK-c promoter in both CpG islands in the liver of chicken fetuses. Lower histone H3 as well as higher H3ac and H3K27me3 levels at both CpG islands were related to the increased binding of CREB to the nearby promoter regions in the fast-growing breed, which was accompanied by enhanced PEPCK-c mRNA expression. The lower density of H3 indicates a more relaxed chromatin structure, which allows transcription factors to bind to the promoter and activate gene transcription. The H3ac is usually associated with transcriptional activation, whereas the H3K27me3 is usually associated with transcriptional repression (Berke et al., 2012). However, the above notion is obtained primarily from in vitro studies; the situation in vivo appears to be more complex. For instance, a gestational high-fat diet resulted in an increased transcription rate of PEPCK-c, which was associated with lower H3ac and H3K27me3 on the PEPCK-c promoter in the livers of rat offspring (Strakovsky et al., 2011). The increase in H3K27me3 on the myostatin (MSTN) promoter was correlated with the higher expression of MSTN mRNA in pigs (Liu et al., 2011). In the present study, the increased H3K27 methylation and H3 acetylation at the CpG island 1 of PEPCK-c promoter were related to the reduced DNA methylation at this CpG island, which marks the gene activation. Functional cross-talks among DNA methylation and different histone codes are complex, and the effect of one histone modification can be positively or negatively influenced by other adjacent or additional modifications (Winter and Fischle, 2010). Therefore, although our results implicate the involvement of epigenetic mechanisms in the breed disparity of PEPCK-c expression in the liver of chicken fetuses, the role of any specific histone markers in the regulation of hepatic PEPCK-c transcription should be investigated in a specific scenario taking other epigenetic modifications and regulatory transcriptional machinery into consideration.

In conclusion, our results indicate that the genetic selection for growth rate in broiler chickens results in breed-specific differences in the epigenetic modifications on the PEPCK-c gene promoter in the liver; these modifications might account for the breed-dependent expression of PEPCK-c in broiler fetuses during the late period of embryonic development. Because hepatic gluconeogenesis is associated with hatching success and posthatching performance, it is worthwhile to investigate whether the epigenetic marks detected on the PEPCK-c gene promoter may serve as potential molecular markers for broiler breeding.

ACKNOWLEDGMENTS

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