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Effect of Feeding Conditions on the Methylation Status of Fatp1 Gene in Chicken Breast Muscle

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Feeding conditions; chicken; FATP1; meat quality; intramuscular fat.

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ABSTRACT

The objective of this work was to investigate the effect of feeding conditions on methylation status of FATP1 gene, which is an important candidate gene of Intramuscular fat and important indicator of chicken meat quality. We selected Daninghe (DNH) and Qingjiaoma (QJM) chickens under scatter-feeding and captivity-feeding conditions as experimental animals, and detected the methylation status of FATP1 genes in chicken breast muscle using Bisulfite Sequencing PCR method. The results showed that the methylation level of FATP1 in scatter-fed chicken was lower than in captivity-fed conditions in DNH and QIM chicken breast tissues; DNA methylation in the promoter and exon1 region was demonstrated to negatively regulate the expression of the FATP1 gene. These results suggested that feeding conditions affect the methylation status and expression level of FATP1, thereby affecting the Intramuscular fat content in DNH and QJM chicken breast muscle.

INTRODUCTION

Chicken meat quality traits are complicated traits that are affected by many factors, such as breed, sex and feeding conditions (Musa *et al*., 2006; HuiFeng *et al*., 2014). Intramuscular fat (IMF) content is an important indicator of chicken meat quality (Li *et al*., 2013), as is the juiciness and flavor of the meat (Murray *et al*., 2004), and these were affected by feeding conditions (HuiFeng *et al*., 2014).

FATP1 is one of the important candidate genes of IMF (Bong *et al*., 2012; Juan Wang *et al*., 2012), which involved many biological processes, such as fatty acids uptake and storage as triglycerides (TGs), extra- and intracellular lipid metabolism, and TG biochemical synthesis and storage (Stahl, 2004; García-Martínez *et al*., 2005; Yuan *et al*., 2012). The polymorphisms of FATP1 gene is associated with chicken carcass traits in Chinese meat-type quality chicken populations and the meat quality of breast muscle (Wang *et al*., 2010).

As an important epigenetic modification, DNA methylation in the promoter is often negatively associated with gene expression, whereas the methylation status in the body of the same gene might be positively related to gene expression (Jones, 2012; Kulis *et al*., 2013).

In this study, to investigate how feeding conditions affect chicken meat quality, we detected the methylation level of the promoter, exon 1 region (–559 to +146 bp) and the mRNA expression of the FATP1 gene. Our finding suggests that feeding conditions affect the methylation status of FATP1 gene, thereby affecting the content of IMF in DNH and QJM chicken breast muscle.

MATERIALS AND METHODS

Ethics Statement

Animal experiments were reviewed and approved by the Research Ethics Committee and the Animal Ethical Committee of the Chongqing Academy of Animal Sciences. The experiment was performed according to the regulations and guidelines established by the Ministry of Science and Technology of the People's Republic of China (Approval number: 2006–398).

Experimental animals

In this study, five-month-old DNH and QJM chicken were selected as experimental animals. The two populations were separated into four groups: DNH chicken under scatter-feeding conditions (SFDNH), DNH chicken under captivity-feeding conditions (CFDNH), QJM chicken under scatter-feeding conditions (SFQJM) and QJM chicken under captivity-feeding conditions (CFQJM), each group size was 300-500. These animals were reared on the same diet at the breeding base in Wulong County, Chongqing, China. The basal diet, water and feeding conditions were the same as those in our previous study (HuiFeng *et al*., 2014; Gao *et al*., 2015). Five chickens were randomly selected from SFDNH, SFQJM, CFDNH and CFQJM groups, respectively. In total, twenty chickens were used in further study.

DNA Extraction and Bisulfate Modification

The left breast muscle tissues of twenty chickens from the four groups that were used for RNA-seq and Real-time PCR in our previous study (Gao *et al*., 2015), were used to study the DNA methylation level of FATP1 gene. Genomic DNA from each sample was extracted from the chicken breast muscle tissues using the DNeasy Blood & Tissue Kit (Qiagen, Dusseldorf, Germany), and treated with the EpiTect Plus Bisulfite Kit (Qiagen), following the manufacturer's instructions. The quality of the genomic DNA was assessed on a NanoVue spectrophotometer (GE LifeSciences, Piscataway, NJ, USA).

The promoter regulation region (–559 to +1 bp), exon1 (+1 to +148 bp), containing one CpG island (–621 to +491 bp) and 82 methylation sites of FATP1 (Picture 1), was amplified from the modified genomic DNA by bisulfte sequencing PCR (BSP). The specific bisulfite sequencing PCR primers for the target region were designed using Methyl Primer Express Software v1. 0 (Applied Biosystems Inc., Foster City, CA, USA) with the sequence of FATP1 (GenBank Accession NW_003779439.1). The FAPT1 primer (forward primer: 5′-GAATTAAGGTTAAAAAGAAGTTTTTT-3′, reverse primer: 5′- TTTACAAATAACCCTCAACAAC-3′) encompassed the promoter and exon 1 region (–500 to +100 bp), which harbors one CpG site. We predicted CpG islands using the Methprimer website (http://www.urogene.org/methprimer/) (Figure 1).

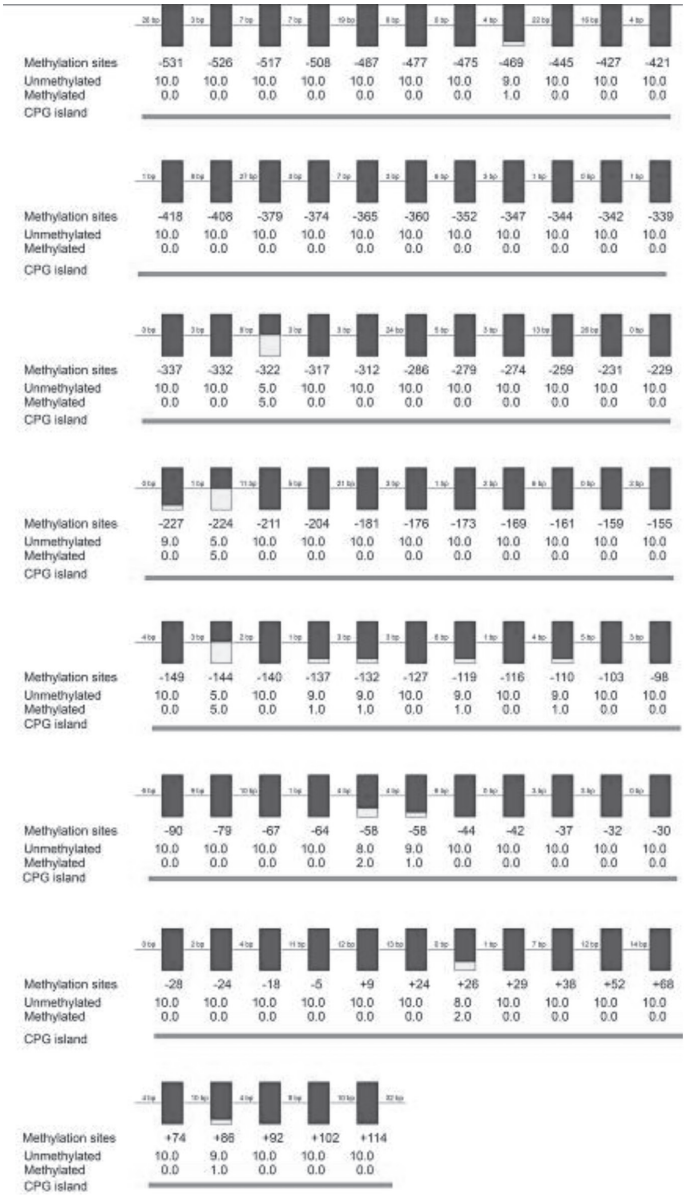

Figure 1 – The mean methylation level of the FATP1 gene at each methylation site in DNH and QJM chickens

The PCR reaction system comprised: 15μL of PCR Mixture 2 × Mix, 1 μL of forward primer (10 μM), 1μL of reverse primer (10 μM), 3μL of DNA template (1 μg/μL), and 10 μL of ddH2O in a total volume of 30μL. The PCR amplification reaction was carried out as follows: hot-start of 94°C for 3 min; 40 cycles of 94°C for 1 min, 58.4°C for 50 s, and 72°C for 30 s; and an extension step of 72°C for 3 min. The amplified PCR prod-

ucts were purified, ligated into the pEASY-T1-vector (Trans, Beijing, China) and transformed into Trans-T1 competent Escherichia coli cells (Trans, Beijing, China). Ten ampicillin-resistant colonies per sample were sub-cultured for plasmid extraction and sequencing.

Statistical Analyses

The differences in methylation and mRNA expression among the four groups were analyzed using Student's t-test. Extremely significant differences and significant differences were considered significant at $p<0.01$ and $p<0.05$, respectively. The relationship between methylation and mRNA expression levels were assessed using Pearson's correlation coefficients. We employed 2 × 2 factor analysis models by the JMP 8 (SAS Inst., Inc., Cary, NC, USA) to evaluate the degree of influence that feeding conditions and breed had on the methylation and expression level of FATP1 gene in chicken breast muscle; the models were described in a previous study (Gao *et al*., 2015).

RESULTS

In order to investigate how feeding conditions affect meat quality, we selected SFDNH, CFDNH, SFQJM and CFQJM chicken as experimental animals, and detected the methylation status of FATP1 genes of each group using BSP method. As shown in figure 2, the methylation level of the FATP1 gene in the scatter-feeding groups was lower or much lower ($p<0.01$) than in the captivity-feeding groups of DNH and QJM chicken (Figure 2), which suggested that the feeding conditions affected the methylation of the FATP1 gene.

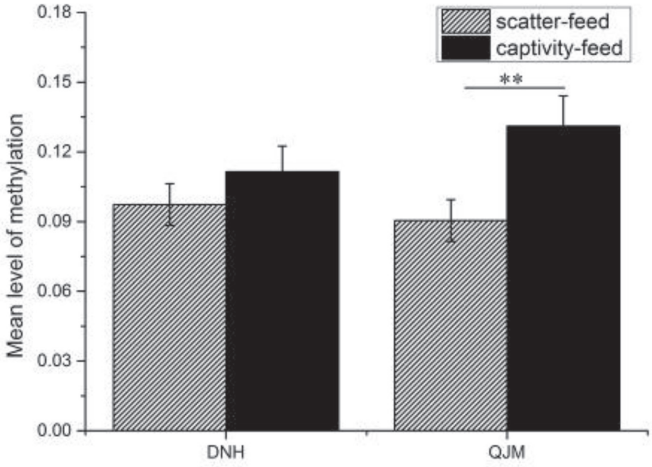

Figure 2 – Mean methylation level of the FATP1 gene in SFDNH, CFDNH, SFQJM and CFQJM chickens

However, there were no differences between the two breeds under scatter-feed or captivity-feed conditions.

Moreover, we utilized a 2 × 2 factor analysis model to evaluate the degree by which feeding conditions and breed influence the methylation and expression level of FATP1 gene in chicken breast muscle, the results showed that the DNA methylation level of the FATP1 gene was significantly associated with the feeding conditions ($p=0.0083$), but not breed ($p=0.74$).

Our previous study in DNH and QJM chicken also showed that the feeding conditions, but not the breed, affected the mRNA expression of FATP1 (HuiFeng *et al*., 2014). We extracted the DNA from the same samples to investigate the DNA methylation of FATP1 gene in the four groups. The Pearson correlation coefficients for methylation and mRNA levels were –0.80, –0.61, –0.62 and –0.77 for SFDNH, CFDNH, SFQJM and CFQJM groups, respectively, which suggested that the methylation status of the –559- to +146-bp region has a negative effect on the expression of the FATP1 gene.

DISCUSSION

DNH chicken is a well-known Chinese egg and meat dual-function local chicken breed, and QJM chicken is bred for its meat (Chen *et al*., 2004). Previous studies show that IMF content in DNH chickens or QJM chickens under captivity feeding condition is significantly higher than scatter feeding condition (Hui Feng *et al*., 2014). In this study, we found that the methylation level of FATP1 gene in DNH chickens or QJM chickens under scatter feeding condition were higher than captivity feeding condition. Methylation status of FATP1 gene may be associated with the content of IMF in chicken breast muscle tissues.

Numerous studies have indicated that DNA methylation involved in many biological processes, such as cell differentiation, gene expression regulation, X chromosome inactivation, genomic imprinting, chromatin modification, cancer development and embryo development biological processes (Bock *et al*., 2012; Jones, 2012; Rigal *et al*., 2012; Akhavan-Niaki and Samadani, 2013; Smith and Meissner, 2013; Vu *et al*., 2013; Tannan *et al*., 2014). In this study, the methylation status of the promoter and exon1 region of FATP1 gene showed a negative relationship with expression of the FATP1 gene, which is consistent with the previous studies that show DNA methylation in the promoter negatively associated with gene expression(Jones, 2012; Kulis *et al*., 2013).

In conclusion, our results showed that feeding conditions affect the methylation status and expression level of the FATP1 gene. The methylation status of the

promoter and exon1 region of FATP1 gene showed a negative relationship with expression of the FATP1 gene. We speculated that feeding conditions affect the methylation of FATP1 gene, methylation affected the expression of FATP1 gene, thereby affecting the content of IMF in DNH and QJM chicken breast muscle.

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