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Developmental Changes of the H-FABP mRNA Expression in Hainan Black Goat

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Abstract: This study investigated the H-FABP mRNA expression levels in Hainan Black goats. Males from 1-4 months of age beyond adult animals were selected. Three animals of each age were slaughtered to collect samples from heart skeletal muscle (Longissimus dorsi muscle, psoas major muscle, biceps femoris muscle) adipose tissue liver and kidney. The total RNA was obtained to investigate the H-FABP mRNA expression levels by real-time PCR. The results showed that the H-FABP mRNA expression levels in heart and skeletal muscle have the same basic trends at different growth periods. The highest H-FABP mRNA expression in heart and skeletal muscle appeared at 2 months of age and it was extreme higher than those at other ages ($p<0.01$). The lowest H-FABP mRNA expression level in these tissues were at 1 month old. H-FABP mRNA expression level in adipose tissue was different from those in heart and skeletal muscle. The highest H-FABP mRNA expression in adipose tissue appeared at adult age and its expression was extreme higher than those in younger ages ($p<0.01$). In addition, the H-FABP mRNA expression pattern in liver was different from the other tissues. The expression in the liver at 1 month of age was significantly higher than those at 2-4 months of age. However, the highest level occurred at adult age. The differences in H-FABP mRNA expression level at 3 and 4 months old were not significant in liver, heart, skeletal muscle and adipose tissue. On the other hand, H-FABP mRNA expression level in heart was the highest and significantly higher than other tissues at all ages. But, H-FABP mRNA expression level in liver was extreme higher than those in skeletal muscle and adipose tissue at 1 month old age ($p<0.01$). Besides, H-FABP mRNA expression in adipose tissue appears to increase as the age increase.

Key words: Goat, H-FABP, mRNA, real-time quantitative PCR, China

INTRODUCTION

The Heart Fatty Acid-Binding Protein gene (*H-FABP*) is regarded as one candidate gene that affects Intramuscular Fat (IMF) deposition and generally thought to be a major factor that influences meat quality of livestock (Chmurzynska, 2006). Genetic variations in *H-FABP* gene loci have been associated with intramuscular fat in pigs (Gerbens *et al*., 1999). It is known that intramuscular fat content is an important aspect of meat quality in domestic animals because it is positively correlated with palatability. The studies showed that the H-FABP mRNA expression is high in the heart and skeletal muscle and low in adipose tissue, kidney and brain. The expression of H-FABP mRNA in adipose tissue of adult pigs was 8.5% of that in heart and 30% of that in skeletal muscle and the H-FABP mRNA level was more than 10% of that of adipocyte fatty acid binding protein mRNA in adipose tissue (Li *et al*., 2007). Especially, its most abundant in adult heart (Robert *et al*., 1987). H-FABP has been shown to be involved in fatty acid uptake and utilization in the heart and skeletal muscle as demonstrated by H-FABP deficient mice (Binas *et al*., 1999, 2003). There are many articles about *H-FABP* genes of humans, rodents, mice, chicken and pigs but rarely about any goat. Hainan Black goat is one of the numerous meat goat breeds and is reared in Southeast China. It is the well-known breed due to not only their tolerance of crushed feed and the local wet weather but also to their delicious meat flavor.

The objective of this investigation was to study the developmental regulation of H-FABP mRNA expression in tissues of heart, skeletal muscle, adipose tissue, kidney and liver during different growth periods in Hainan Black goat. The purposes were finding out the differential expression of *H-FABP* gene in goats at different growth periods and different tissues whether exist or not the stable mRNA expression variation in the gene and thereby affecting the genes protein content which ultimately affects the differences in meat quality in different growth periods of goats. This will provide the theoretical basis for the further studies on improvement of meat quality.

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MATERIALS AND METHODS

Tissue harvest: Goats of Hainan Black breed males from 1-4 months of age beyond adult animals were obtained from China Tropical Agriculture Research Institute. Three animals of each age (15 in total) were slaughtered to collect samples from heart, skeletal muscle (Longissimus dorsi muscle, psoas major muscle, biceps femoris muscle), adipose tissue and liver. After collecting the samples were stored at -80°C for total RNA analysis.

Major equipment and reagent: Maxwell 16 total RNA purification kit and AMV RTase were purchased from Promega (USA). SYBR Premix Ex Taq was purchased from Dalian Bao Biotech Co. Nucleic acid extraction device (Maxwell 16) was from Promega. PCR equipment was from Biometra (Germany). Gel image system 120 was from Kodak.

Total RNA extraction and reverse transcription: Total RNA from tissue was isolated using Trizol (Invitrogen) following the manufacturer's instructions. The quantity and quality of RNA were evaluated by agarose gel and 1 μg of RNA was used in each reverse transcription. Reverse transcription was performed using M-MLV reverse transcriptase (Invitrogen). The conditions for reverse transcription were 65°C for 5 min, 37°C for 50 min and 70°C for 15 min.

Primer design: Primers were designed using Primer 3.0 (http://frodo.wi.mit.edu/) according to the published sequences of H-FABP and β-Actin mRNA for goats at GenBank (*H-FABP* is the target gene and the *β-actin* is reference gene). Relevent information was shown in Table 1.

SYBR green real-time PCR: Reaction were performed with Peaplex[4] quantitative PCR equipment (Eppendorf). The reaction mix (25 μL) contained 12.5 μL of SYBR Premix Ex Taq, 1 μL of primers (10 μmolL^{-1}), 2 μL of DNA template, 9.5 μL of ddH_2O. The thermal cycle parameter was as the following preheat at 95°C for 30 sec, 45 cycles of denaturation at 95°C for 30 sec, 45 cycles of denaturation at 95°C for sec, annealing/extension 20 sec (Table 1) and extension at 72°C for 15 sec. During melting curve analysis, temperature was gradually raised from 60-95°C at a rate of 0.875°C/30 sec. Each time internal reference and unknown samples were assessed with triplicates and one No Template Control (NTC) was used.

Statistical analysis: In this experiment, the samples of heart, liver, adipose tissue and skeletal muscle were the experimental while those of kidney were the calibration samples. The realplex software calculated the Ct of the target and reference gene. This method is used to accurately quantify the amount of the initial material, especially when the amount of the initial material is often limited. In this study, the $2^{-\Delta\Delta CT}$ method (Livak) was used to normalize of the quantitative data. Data were described as c±sd and statistically analyzed using SPSS11.5 for Windows software. The same tissue expression level in different growth periods and the different tissue expression level in the same growth periods were analyzed by one-way ANOVA and independent sample t-test, respectively. Significant and extreme differences were set at $p<0.05$ and $p<0.01$, respectively.

RESULTS AND DISCUSSION

Heart and skeletal muscle mRNA expression of H-FABP in different growth periods: As shown in Fig. 1, the H-FABP mRNA expression levels in heart and skeletal muscle showed the same basic trends according to different growth periods of males Hainan Black breed. In these tissues, H-FABP mRNA is highly expressed at 2 months of age and in adult age (after 12 months). The H-FABP mRNA expression in heart and skeletal muscle at 2 months of age was superior to observed in others ages ($p<0.01$). The lowest H-FABP mRNA expression occurred at 1 month old. The pattern of H-FABP mRNA expression in heart and skeletal muscle was 2 months>adult age (12 months) >3 and 4 months>1 month. Among the skeletal muscles, the H-FABP mRNA expression was higher in longissimus dorsi muscle than in psoas major muscle and biceps femoris muscle.

Liver and adipose tissue expression of H-FABP mRNA in different growth periods: As shown in Fig. 2, the H-FABP mRNA expression levels in adipose tissue were different from heart and skeletal muscle in different growth

Table 1: Real-time RT-PCR primer information

Gene name	Primers sequence	Product size (bp)	Annealing temperature (°C)	GenBank accession No.
H-FABP	F: 5'TGAGACCACGGCAGATGA3'	115	55.0	FJ844408
	R: 5'AACTATTTCCCGCACAAG3'			
ACTB β-actin	F: 5'CACGGTGCCCATCTACGA3'	157	56.2	AF481159
	R: 5'CTTGATGTCACGGACGATTT3'			

ACTB (β-actin) was the internal reference

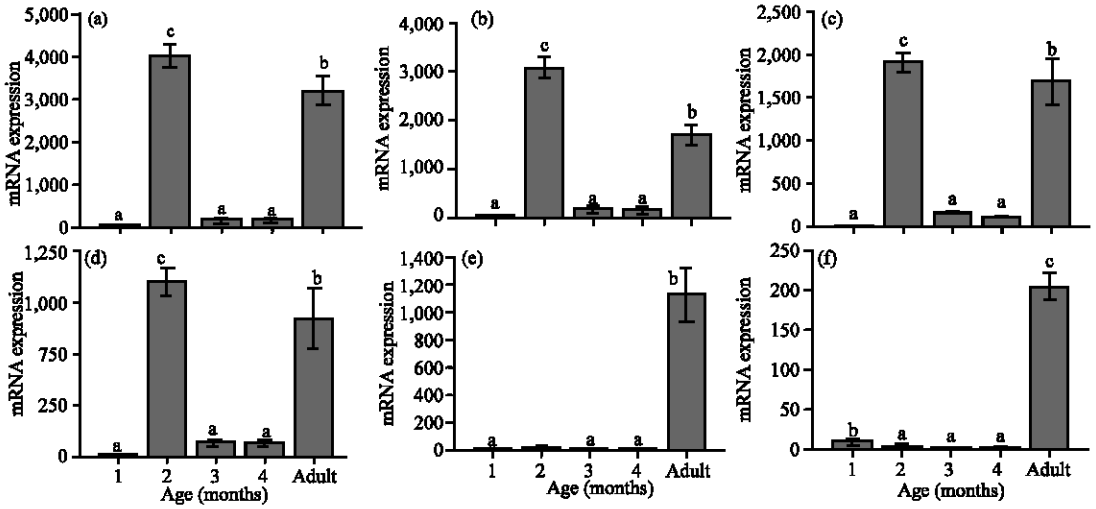

Fig. 1: mRNA expression of H-FABP in different growth periods: a) Heart; b) LDM; c) PMM; d) BFM; e) AT; f) liver; LDM: Longissimus Dorsi Muscle; PMM: Psoas Major Muscle; BFM: Biceps Femoris Muscle; At: Adipose Tissue; The bars indicate average ±sd (n = 3 goats); averages followed by the different letters mean extreme significant difference ($p<0.01$)

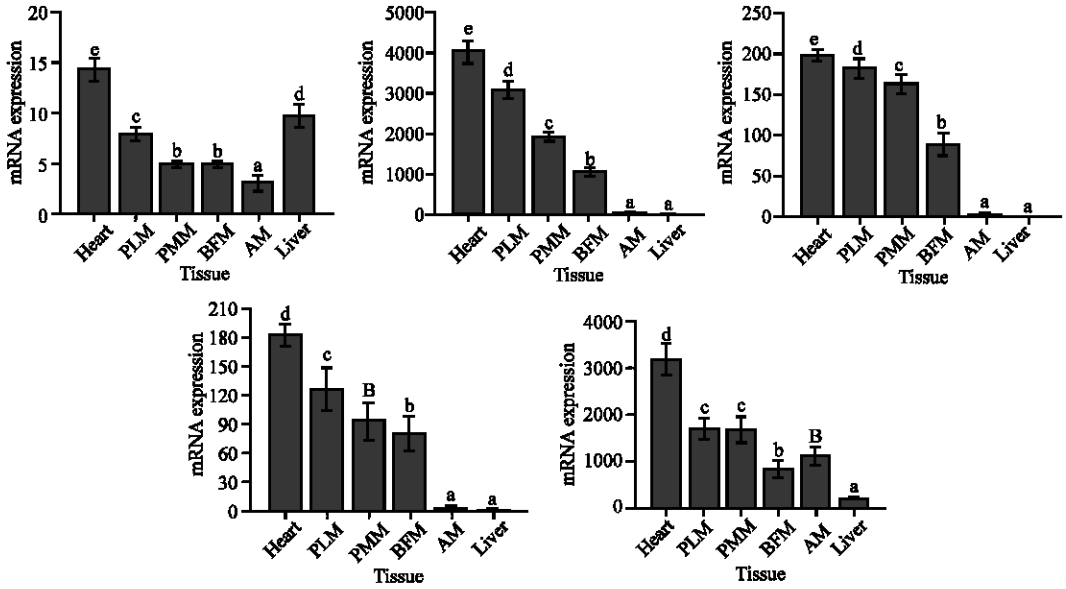

Fig. 2: Different tissue expression of H-FABP mRNA in same growth periods: a) 1 month; b) 2 months; c) 3 months; d) 4 months; e) Adult; the bars indicate averages±sd (n = 3 goats); averages followed by different letters mean extreme significant difference ($p<0.01$); averages followed by the same letter but with different case (upper and lower) mean significant differences ($p<0.05$)

periods. The highest H-FABP mRNA expression in adipose tissue occurred in adult age (after 12 months of age) and this expression was extreme higher than in the others ages ($p<0.01$). The pattern of H-FABP mRNA expression in adipose tissue was 12>2>3 months and 4 months>1 month. In addition, the H-FABP mRNA expression pattern in liver was different from the other tissue. This expression at 1 month of age was significantly higher than those at 2-4 months ($p<0.05$); however, was lower than that in adult age. The pattern of H-FABP mRNA expression in liver was adult age>1 month>2 and 3 months>4 months.

Different tissue expression of H-FABP mRNA in same growth periods: As shown in Fig. 2, the level of H-FABP mRNA expression in heart was the highest and extreme higher than those in other tissue in all ages ($p<0.01$). In addition, the trend of H-FABP mRNA expression level

according to tissue was heart >skeletal muscle >adipose tissue >liver. The lowest level was observed in liver at 2-4 months of age despite the expression in this tissue was extreme higher than in skeletal muscle and adipose tissue at 1 month old age ($p<0.01$). Besides H-FABP mRNA expression in adipose tissue appears to increase suddenly and be significant higher than in biceps femoris muscle. On the other hand, the expression level of H-FABP mRNA in adipose tissue increased as the age increase.

In this study, researchers characterized the expression of H-FABP mRNA in heart, skeletal muscle, adipose tissue and liver during the development of goats of Hainan Black breed by real-time PCR. This study is the first to quantitatively compare goat H-FABP mRNA under different growth periods and different tissue. It was demonstrated that H-FABP is strongly expressed in heart and skeletal muscle and that there is extreme or significant difference among the tissues evaluated. The levels of expression in the same tissue also are modified according to different growth periods. Previous researches on this subject were mainly focused on humans, rodents, pigs, chicken and sheep. Surprisingly, compared with previous studies (Robert *et al*., 1987; Armstrong *et al*., 1990; Ding *et al*., 1999), H-FABP mRNA was also detected in liver at a moderate level at 1 months of age and reached the highest level in adult age (>12 months). H-FABP mRNA in adipose tissue increased as the age increase.

CONCLUSION

It is interesting to note that the H-FABP mRNA expression in heart, skeletal muscle, adipose tissue and liver was very low at 3 and 4 months old when compared with those at 2 months and adult age. H-FABP mRNA expression level difference was not significant in liver, heart, skeletal muscle, adipose tissue between 3-4-months old age. However, the level of expression at these ages in liver were extreme higher than those observed in skeletal muscle and adipose tissue at 1 month old age ($p<0.01$). It is notable that the expression level of H-FABP mRNA in adipose tissue and liver reached the highest at adult age. Tyra *et al*. (2011) reported the *H-FABP* gene expression profile in skeletal muscles and liver during ontogenesis in various breeds of pigs. They showed that the expression of this gene in muscles did not change with age or breed. However, the expression levels in liver were higher in young pigs (60 and 90 days). *H-FABP* genes are strongly related to the development and function of fat tissue in pigs. In mouse white adipose tissue, keratinocyte fatty acid binding protein (mal 1) mRNA was moderately expressed and H-FABP mRNA was very lowly expressed whereas in brown adipose tissue this later was highly expressed and mal 1 mRNA was very lowly expressed (Maeda *et al*., 2003).

Further studies are necessary to know why this gene has differential expression at different developmental stages and whether these different expressions are stable as well as the expression patterns in other ages do not evaluated here. This experiment provides a reference to studies of gene expression and regulation mechanism on traits related to meat quality in Hainan Black goats.

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