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Investigation of SNPs in FABP3 and FABP4 Genes and Their Possible Relationships with Fatty Acid Composition in Broiler

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ABSTRACT There is limited information of the genetic effect for fatty acid composition in chicken meat. This study assessed the association of FABP3 and FABP4 genes affecting fatty acid composition in broilers. Two single nucleotide polymorphisms (SNPs) were detected in FABP3 gene and five SNPs were identified in FABP4 gene. The SNPs located in intron 1 and exon 1 of FABP3 and FABP4, respectively, were used for genotyping using PCR-RFLP method. The SNPg.285C >T in FABP4 showed suggestive association with high arachidonic acid (C20 : 4) in CT genotypes (P = 0.068). However, the SNP g.508C > T in FABP3 showed no significant associations with fatty acid composition. These results are the first report to investigate the SNPs in FABP3 and FABP4 genes and their associations with fatty acid composition, although we only found the possible association of FABP4 SNP with fatty acid composition. These results should provide valuable information for further investigation of the genes affecting fatty acid composition in chicken.

(Key words : FABP3, FABP4, fatty acid composition, chicken)

INTRODUCTION

Meat is an important food for human. When people consume meat, they also eat fats in the meat. Therefore the fat composition, especially fatty acids (FAs), is known to be very important for human health. Previously, high intake of saturated FA (SFA) can elevate plasma cholesterol, which contributes to cardiovascular disease (Bronte et al., 1956). SFAs such as lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0) are considered to have the most harmful cardiovascular effects (Keys et al., 1974). However, high intake of polyunsaturated FA (PUFA) and monounsaturated FA (MUFA) increases hepatic low density lipoprotein (LDL) receptor activity, which leads to the decrease in the circulating concentration of LDL-cholesterol (Woolett et al., 1992). Many consumers consider that red meat is unhealthy because of high SFA and cholesterol levels. Therefore, the replacement of red meat with chicken (white) meat is becoming popular due to consumers' demands for healthy foods. Previous study indicated that diet replacement from red meat to the chicken meat can decrease apolipoprotein B and total cholesterol levels in microalbuminuric (Gross et al., 2002). This may due to the different PUFA contents between chicken meat and red meat (beef). Chicken meat also has a lower portion of saturated and higher PUFA especially in long-chain omega 3 PUFA eicosapentaenoic and docosahexaenoic acids than beef (Almeida et al., 2006). Therefore, fatty acid (FA) composition in chicken meat is very important factor for the consumers' point of view.

The selection of animals having desired phenotypes including desired FA composition can be achieved using molecular techniques including candidate gene approach. One of the strong candidate genes is the FABP gene family. FABPs belong to the member of a superfamily of lipid-binding protein. Based on the tissue-specific distribution, FABPs divided into nine different tissues (Chmurzynska, 2006). Two of them are FAPB3 and FABP4 which also well known as H-FABP and A-FABP, respectively. FABPs act mainly as FA transporters in the metabolic pathway (Estelle et al., 2009), where the peroxisome proliferators-activated receptor (PPAR) family members cooperatively participate as transcription factors. The knock-out mouse study indicated that FAPB3 gene is important for FA transportation and metabolism (Binas et al., 1999).

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Many studies have been carried out for the association with FABP polymorphisms and fat-related traits in chicken. The FABP4 overexpression adipocytes affect the lipid metabolism through the PPARγ pathway in Arbor Acres chicken (Shi et al., 2010). Markers at the FABP3 and FABP4 genes were associated with IMF content in a population of male Beijing-You chickens (Ye et al., 2010) and in two Chinese chicken breeds Beijingyou and Jingxing (Li et al., 2008). Also, Luo et al. (2007) suggested that the SNP in first exon of FABP4 had effect for the differences among abdominal fat percentage, subcutaneous fat thickness and intramuscular fat contents in breast muscle. However, none of study has been reported in the polymorphisms of FABP gene family and their associated with FA composition in chicken. Therefore, the aim of this study is to identify SNPs in H-FABP and A-FABP genes and their possible associations with fatty acid composition in broiler chicken.

MATERIALS AND METHODS

1. Animals and Samples

Ninety five Ross broilers were used in this study. They were reared under the same feeding conditions until 1.6 kg of slaughter weight at 28 days age. Tissues from thigh muscle were sampled for genomic DNA isolation and FA composition analysis.

2. FA Analysis

Total lipid in each sample was extracted using chloroform-methanol (2:1, v/v) according to the procedure of Folch et al. (1957). FA methyl esters were prepared from the extracted lipids with BF3-methanol (Sigma-Aldrich, St. Louis, MO, USA) and separated on a HP-6890N gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) as described previously (Jeon et al., 2010). A split inlet (split ratio, 50:1) was used to inject samples into a 30 m × 0.25 mm × 0.25 μm Omegawax 320 capillary column (Supelco, Bellefonte, PA, USA). The oven temperature regimen consisted of 150℃ for 3 min, an increase to 180℃ at 2.5℃/min, 180℃ for 5 min, an increase to 220℃ at 2.5℃/min, and 220℃ for 25 min. The inlet temperature was 210℃. Air was the carrier gas at a constant flow of 0.7 mL/min.

3. DNA Extraction and Genotyping

Genomic DNAs were extracted from muscle samples and isolated by 20 mg/mL proteinase K digestion followed by phenol extraction. Primers and polymerase chain reaction (PCR) information for the FABP3 and FABP4 genes were shown in Table 1. The PCR mixture contained 50 ng genomic DNA, 10× buffer mix and 10 mM dNTPs (GenetBio, Chungnam, Korea). Amplifications were performed at 10 min at 94℃, 35 cycles of 30 s at 94℃, 30 s at the annealing temperature, and 30 s at 72℃, and a final extension of 10 min at 72℃ using either a GeneAmp PCR system 2700 (Applied Biosystems, Franklin Lakes, NJ, USA) or a C1000^{TM} Thermal Cycler (BioRad Laboratories, Hercules, CA, USA). The PCR products were visualized in 1.5% standard agarose gels stained with ethidium bromide (GenetBio). Each PCR fragment was purified using an AccuPrep PCR Purification Kit (Bioneer, Daejeon, Korea). Purified PCR products were sequenced using the same primers for PCR reaction in a model 3730 XL automated DNA Sequencer (Applied Biosystems). The DNA sequences were analyzed with the BioEdit program ver. 7.00 (Tom Hall, Ibis Therapeutics, California, USA) and the single nucleotide polymorphisms (SNPs) were confirmed based on the electrophoregram results. The SNPs in FABP3 and FABP4 genes were genotyped by the PCR-restriction fragment length polymorphism (PCR-RFLP) method (Table 1). The restriction enzyme digestion was performed in 20 μL reaction volumes with approximately 15 μL of PCR products and 2 units of each restriction enzyme. The digested products were run on 3% agarose gels.

4. Statistical Analyses

The effects of FABP3 and FABP4 genotypes on FA composition traits were tested using the general linear model (GLM) in the SPSS ver. 17.0 program (SPSS, Chicago, IL, USA). In order to test the pair wise differences between the effects of genotype, least significant difference (LSD) test was also performed. Pearson's Chi-square test was used to test the allele and genotype frequency for Hardy-Weinberg equilibrium. The following model was used to test the association of the genotype with FAs composition:

$$Y_i = \mu + G_i + \varepsilon_i$$

Table 1. Primers for PCR amplification and SNP identification in FABP3 and FABP4 genes

Gene	GenBank accession No.	Sequence (5' to 3')	PCR product size (bp)	Annealing temperature	Identified SNP (location)
FABP3	NC_006110	F3-1: ggtgatgcatgaggacattg	460	58℃	g.476G > A (intron 1)
		R3-1: actaccgccttgctcacact			g.508C > T (intron 1)
		F3-2:gaatggtggttctgctct	162	58℃	No SNP identified
		R3-2:tcccccatctaaaatcct			
		F3-3:tgctcacctcctgctctttg	219	58℃	No SNP identified
		F3-3:ccatgagaccacagcatcac			
FABP4	NC_006089	F4-1: tgtgacctactggcaaagga	477	58℃	g.184A > G (UTR)
		R4-1: ttcctcccagtcaagctttc			g.285C > T (exon 1)
		F4-2: gcaattgccttgtctcatcc	714	58℃	g.1533C > T (intron 2)
		R4-2:aactcaccaccagcaggttc			g.1965A > G (exon 3)
		F4-3:attggctcccagtgcataag	361	60℃	g.3547C > T (intron 4)
		F4-3:caaggcccgttctgactaat			

Where, Y is the phenotypic data (FA composition) of sample i, μ is the overall mean, G is the genotype effect of sample i and ε is a random error.

RESULTS AND DISCUSSION

1. FA Composition Profile

Table 2 shows the descriptive statistics for the FA composition in 95 broiler chicken samples used in this study. Fourteen FAs including total SFA, PUFA and MUFA were measured in each meat sample. The results indicated total SFA was lower than MUFA but higher than PUFA. Total SFA contained four FAs, namely myristic acid (C14:0), palmitic acid (C16:0) stearic acid (C18:0) and arachidic acid (C20:0), with an average level of 0.89%, 24.27%, 10.47% and 0.09%, respectively. Total MUFA (C14:1; C16:1; C18:1) and PUFA (C18:2; C18:3; C20:4; C22:6) were calculated by adding each of the three and four FAs, respectively. White meat including chicken meat was considered has lower level of SFA and higher PUFA compared to red meats (Almeida et al., 2006). The high level of PUFA influences the lipid oxidation that affects in meat quality including color, flavor, texture and nutritional value (Tang et al., 2001). The current study also detected the level of total SFA was lower than previous study in beef (Bhuiyan et al., 2009) but higher than pigs (Estelle et al., 2009a). Moreover, these results also indicated having more number of unsaturated FA, which is important to dietary alteration in human that leads to decrease in the circulating concentration of LDL-cholesterol (Woolett et al., 1992). Interestingly, this result detected the rare FA, docosahexaenoic acid (DHA). DHA is essential for infants, especially for growth and functional development of the brain (Horrocks and Yeo, 1999). Plentiful dietary DHA improves learning ability of infants. Dietary and age considered have influencing in the variation of FA composition in broiler (Lopez-Ferrer et al., 2001; Poureslami et al., 2010). Poureslami et al (2010) reported broiler feed with linseed oil and fish oil resulted in increasing C18:2n-6 and C20:4n-6; C22:6n-3, C20:5n-3 and C22:5n-3, respectively. Moreover, the effect of age decreased the level of long-chain n-3 and n-6 PUFA, but gender gave marginal effect to FA composition. This indicates that many factors influence variation of FA composition.

Table 2. Descriptive statistics for fatty acid composition in 95 broilers used in this study

Traits	Mean	SD[4]	Min	Max
Myristic acid (C14:0)	0.89	0.18	0.00	1.19
Myristoleic acid (C14:1)	0.19	0.07	0.04	0.34
Palmitic acid (C16:0)	24.27	1.13	21.89	28.00
Palmitoleic acid (C16:1)	4.58	1.07	1.80	6.76
Stearic acid (C18:0)	10.74	2.53	5.93	20.09
Oleic acid (C18:1)	37.62	3.59	28.26	45.14
Linoleic acid (C18:2)	16.90	1.54	13.37	20.53
α Linolenic acid (C18:3)	0.64	0.15	0.28	1.00
Arachidic acid (C20:0)	0.09	0.03	0.05	0.20
Arachidonic acid (C20:4)	3.49	1.33	1.05	6.37
Docosahexaenoic acid (C22:6)	0.54	0.21	0.14	1.06
SFA[1]	36.00	3.07	30.00	47.22
MUFA[2]	42.41	4.46	30.10	50.66
PUFA[3]	21.59	2.72	15.91	28.52

The superscripts 1, 2, 3 denote total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), respectively. [4]SD is standard deviation of percentage FA composition.

2. Genotyping and Allele Frequency

SNPs were initially identified by direct sequencing of the FABP3 and FABP4 genes using fifteen chicken DNA samples. As the results, two SNPs of FABP3 and five SNPs of FABP4 were identified. Among these, the SNP g.508C > T in FABP3 located in intron 1 and The SNP g.285C > T in FAPB4 located in exon 1 can use for the genotyping using PCR-RFLP. Initially, the 460 bp PCR product of FABP3 was digested using *Nla*III restriction enzyme. Four fragments, 201, 176, 72 and 11 bp, were identified in animals having the CC genotype, and five fragments, 201, 140, 72, 36 and 11 bp, were identified in TT genotype (Fig. 2). It should be noted that the small fragments (less than 100 bp) were not distinguishable in the gel pictures. Similarly, FABP4 PCR product (477 bp) was digested into 235 and 242 bp in CC genotype, but undigested results were observed in animals having TT genotype (477 bp) using *Tag*I restriction enzyme (Fig. 2). The geno

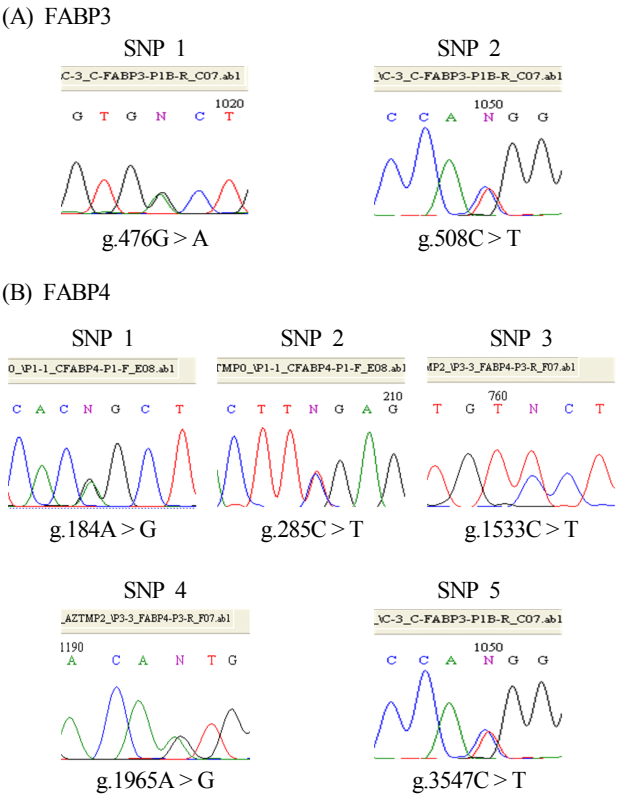

Fig. 1. Electrophoregram results for the identified SNPs in FABP3 (A) and FABP4 (B) genes.

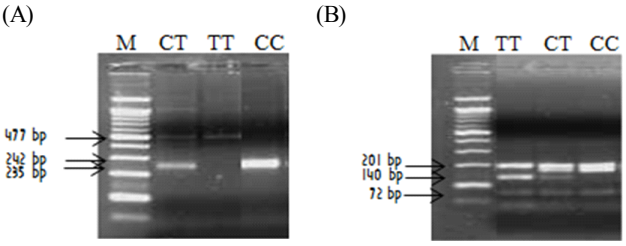

Fig. 2. PCR-RFLP patterns of g.508C > T SNP (digested with *Nla*III) in FABP3 gene (A) and g.285C > T SNP (digested with *Taq*I) in FABP4 (B).

type frequency of both of genes was shown in Table 3. Pearson's Chi-square test was used to test the Hardy-Weinberg equilibrium and the genotypes of FABP3 and FABP4 genes were deviated from Hardy-Weinberg equilibrium (HWE). The deviation from HWE may be due to a variety of causes. Mutation, gene flow, non-random mating (assortative mating), genetic drift and selection are possible factors that may lead to deviate from HWE (Falconer and Mackay, 1996). In a case of this study, we can postulate non-random mating and small

Table 3. The effect of SNPs in FABP3 and FABP4 genes with fatty acid composition in broilers (mean ± SE)

Trait	FABP3			FABP4		
	g.508C > T			g.285C > T		
	CC (0.33)[a]	CT (0.55)	TT (0.12)	CC (0.27)	CT (0.60)	TT (0.13)
Myristic acid (C14:0)	0.88 ± 0.03	0.91 ± 0.03	0.89 ± 0.05	0.93 ± 0.04	0.88 ± 0.02	0.94 ± 0.05
Myristoleic acid (C14:1)	0.20 ± 0.01	0.19 ± 0.01	0.19 ± 0.02	0.20 ± 0.01	0.19 ± 0.01	0.21 ± 0.02
Palmitic acid (C16:0)	24.22 ± 0.20	24.40 ± 0.16	23.78 ± 0.34	24.50 ± 0.22	24.20 ± 0.15	24.13 ± 0.33
Palmitoleic acid (C16:1)	4.58 ± 0.19	4.54 ± 0.15	4.71 ± 0.33	4.67 ± 0.21	4.50 ± 0.14	4.84 ± 0.31
Stearic acid (C18:0)	10.68 ± 0.46	10.92 ± 0.35	10.38 ± 0.77	10.70 ± 0.50	10.86 ± 0.34	10.28 ± 0.74
Oleic acid (C18:1)	37.94 ± 0.64	37.08 ± 0.49	38.81 ± 1.07	37.19 ± 0.70	37.45 ± 0.47	39.38 ± 1.03
Linoleic acid (C18:2)	16.77 ± 0.28	17.05 ± 0.22	16.69 ± 0.47	16.94 ± 0.30	17.00 ± 0.20	16.35 ± 0.45
α Linolenic acid (C18:3)	0.64 ± 0.03	0.64 ± 0.02	0.63 ± 0.05	0.65 ± 0.03	0.63 ± 0.02	0.68 ± 0.04
Arachidic acid (C20:0)	0.09 ± 0.005	0.10 ± 0.004	0.08 ± 0.009	0.10 ± 0.01	0.09 ± 0.004	0.10 ± 0.009
Arachidonic acid (C20:4)	3.45 ± 0.24	3.61 ± 0.18	3.31 ± 0.40	3.56 ± 0.26	3.64 ± 0.17	2.67 ± 0.38
Docosahexaenoic acid (C22:6)	0.51 ± 0.04	0.56 ± 0.03	0.54 ± 0.06	0.56 ± 0.41	0.55 ± 0.03	0.43 ± 0.31
SFA[1]	35.87 ± 0.55	36.33 ± 0.43	35.13 ± 0.93	36.45 ± 0.61	36.03 ± 0.41	35.45 ± 0.89
MUFA[2]	42.72 ± 0.80	41.81 ± 0.61	43.71 ± 1.34	42.07 ± 0.87	42.13 ± 0.59	44.42 ± 1.28
PUFA[3]	21.38 ± 0.50	21.86 ± 0.38	21.16 ± 0.82	21.71 ± 0.53	21.82 ± 0.36	20.13 ± 0.78

[a]Genotype frequency.
[1-3]Total SFA: Saturated fatty acid; Total MUFA: Monounsaturated fatty acid; Total PUFA: Polyunsaturated fatty acid.

population size may influence this deviation.

3. Effects of SNP Genotypes with FA Composition

The general linear model was used to analyze the association of polymorphisms in two FABP family genes with FA compositions (Table 3). The LSD test was carried out to investigate the comparisons of the means of each FA composition among genotypes. The investigation of the genotype effects revealed g.285C > T polymorphisms in exon 1 of FABP4 shown have no associated with FA composition. Similarly, the SNP identified in FABP3 had not demonstrated any interaction with FA composition.

In this study, ninety five broilers were genotyped using the SNPs in the FABP3 and FABP4 genes. The objective of this study was to evaluate whether there is potential genetic association between the SNP markers in both genes and FA composition. There is a causal reason for the hypothesis because FABP genes encoding the protein that are involved in FA transfer to acceptor membranes by direct interaction with the phospholipid bilayer or by an aqueous diffusion-mediated process (Hsu and Storch, 1996). Another study supported that the overexpression of FABP plasma membrane group in mammalian tissues can increase FA transport (Clarke et al., 2004). They reported, in mammalian, FABP plasma membrane overexpression increased the rates of palmitate transport across the sarcolemma, an effect that was independent of any changes in FAT/CD36. In the present study, a novel g.285C > T SNP in the third exon of the chicken FABP4 gene has been detected and shown the concentration of arachidonic acid (C20:4) increased to 3.64% in heterozygote genotype ($P < 0.05$) and decreased to 2.6% in TT genotype compare to CT genotype ($P < 0.021$) even though overall effect of those genotypes did not significant ($P = 0.068$). Therefore, there is limi-

tation in determining the association of the SNP with arachidonic acid (C20:4) confidently. Similarily, docosahexaenoic acid (C22:6; $P = 0.068$) and PUFA ($P = 0.051$) have suggestive associations. Moreover, the results of GLM analysis indicated the SNP g.508C > T in FABP3 had no association in all of FA composition traits.

CONCLUSION

In this study, two SNPs in FABP3 and five SNPs in FABP4 genes were identified in broilers and their possible associations with fatty acid composition in thigh muscle tissue were investigated. Even though there were no association between the SNPs in both FABP3 and FABP4 with FA composition, the suggestive significant results for FABP4 and arachidonic acid (C20:4) have been identified in this study. The results presented here can provide valuable information for further investigation of the genes affecting fatty acid composition in chicken.

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