Identification of single nucleotide polymorphism of gen insuline-like growth factor binding protein 2 on growth of native chicken[1]

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ABSTRACT: Insulin-like growth factor binding protein 2 (IGFBP 2) regulates a broad spectrum of biological activities involved in growth, development, and differentiation. Single nucleotide polymorphisms (SNP) of IGFBP 2 was selected to identify the genotype of black and white colors feather of native chicken with restriction fragment length polymorphism. Trait data from total 48 black and white colors feathers chicken from random population was recorded. The association of the SNP with the weekly body weight was analyzed. The results of the factor analysis indicated that the genotypes of the SNP were only significantly associated with body weight at DOC, and at 7, 14, and 21 day of age. The frequency of restriction enzyme C/T alleles in the black color was 0.48 (C) and 0, 52 (T), for the white color 0.41 (C) and 0.59 (T), and for black and white 0.45 (C) and 0.55 (T). Genotype among black, white, and black-white colors feather did not differ from the expected Hardy-Weinberg equilibrium.

Key words: chicken insulin-like factor binding protein 2 gene, single nucleotide polymorphism, growth

INTRODUCTION

Consumers' preference for colored-feather and slow-growing meat-type chickens is growing in certain regions of the world (Rizzi et al., 2007). In general, colored-feather and slow-growing meat-type chickens are mainly bred from the native breeds (Zhao, 2007) with poor feed conversion. (Yang and Jiang, 2005) The appearance (plumage, skin, combs and so on), meat flavor, and meat texture (Yang and Jiang, 2005) and meat color are the main attributes that attract customers to purchase them (Zhao, 2007).

Body weight of black, black-white and white color native chicken at age 12 week were only 842,9; 797,4, and 747,7 g for black, black-white, and white color respectively (Sri-Sudaryati 2010). Crossing between male native chicken and brown color layer type chicken had increased the body weight of their offspring. Body weight 12 week old of the offspring of crossing between native chicken and Lohmann Brown was 1,007.54 g for male and 958,51 for female, whereas native chicken and Isa Brown were 1,016.63 g for male and 963.03 for female (Kurniawan, 2007).

Single nucleotide polymorphisms (SNP), one base change including deletion, insertion, and substitution, play an important role in the transcription and translation of genes and affect function of protein. A haplotype is a physical arrangement of SNP alleles along a chromosome (Olivier, 2003). Haplotype-based methods offer a powerful approach to disease gene studies based on the association between the single SNP and the haplotypes (Knoblauch et al., 2002)

The genes that are part of the somatotropic axis play a crucial role in the regulation of growth and development of chickens. The identification of genetic polymorphisms in these genes will enable the scientist to evaluate the biological relevance of such polymorphisms and to gain a better understanding of quantitative traits like growth (Nie et al., 2005).

The insulin-like-growth factor (IGF) system is well defined, with profound effects on the growth and differentiation of normal and malignant cells. In biological fluids, IGFs are normally bound to IGF-binding proteins (IGFBPs). There are, at present, seven well characterized mammalian IGFBPs.

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Therefore, IGFBPs act not only as carriers of IGFs, thereby prolonging the half-life of the IGfs2, but also function as modulators of IGF availability and activity (Hwa et al., 1999). the IGFBP2 is sensitive to dietary protein level and may play an important role in the modulate the growth promoting effect of circulating IGF-I by making the IGFIGFBP complex in ruminant and chicken (Kita et al., 2002). The chicken IGFBP2 gene spans approximately 38 kb and is located on chromosome 7 (Schoen et al., 1995). It consists of 4 short exons and 3 long introns, encoding a 275-amino acid polypeptide hormone and is regulated by growth hormones (Schoen et al., 1995).

Forty SNP were identified while scanning 3,578 bp of available sequence of the chicken IGFBP2 gene in 4 divergent populations of Leghorn, White Recessive Rock, Taihe Silkies, and Xinghua chickens by denaturing high-performance liquid chromatography (Nie et al., 2005). The genotype-phenotype association analysis showed that the difference induced by the haplotypes derived from the 5 SNP was more significant than that by the single SNP (Lei, 2005). Li et al. (2006) shown that chicken IGFBP2 gene intron 2 C1032T (accession number AY 326194) polymorphism was associated with growth and body composition traits in an F2 population.

The objectives of the current study were to identify SNP in the IGFBP2 gene, develop PCR-RFLP methods to detect those DNA polymorphisms in black and white native chicken color plumage populations, and evaluate associations between IGFBP2 SNP and trait of growth.

MATERIALS AND METHODS

Chicken Management

Fifty black and 50 white color feather DOC native chickens were used in this study. According the color feather, all of the chicken was housed on litter cage 1 m^2 for 10 chickens. Broiler and Kampung commercial diets were provided in the study. From hatch to 6 week of age, the chicken received a broiler diet (3,100 – 3,200 kcal/kg of ME and ±210 g of CP/kg) and from 7 to 12 wk of age, all birds were fed with Kampung commercial diet (2,050 – 2,150 kcal/kg of ME and ±10 g of CP/kg). All birds had ad libitum access to feed and water. The individually body weight was measured in grams at DOC and at 7, 14, 21, 28, 35, 49, 56, 63, 70, and 84 day of age. Feed consumption and feed conversion were measured weekly. In every cage, five chickens were chooses for genomic DNA, but two white chickens died at the end of the experiment.

Development of PCR-RFLP Assays

Genomic DNA was isolated from venous blood located in EDTA. A PCR was carried out with 50 ng of genomic DNA from the random black and white Kampung chicken to investigate sequence polymorphism of the intron 2 region of the IGFBP2 gene. Primer (5′ - TTTGGTTGAGTCCTAGGCTTG - 3′, 5′ - GGCGTACTACACTGCAGAGG - 3′) was aimed to amplify the fragment (527 bp) of the chicken IGFBP2 gene containing SNP C1032T with accession number AY 326194.

The PCR was performed in a final volume of 20 µL megamix-blue, 1 µL F primer, 1 µL R primer, 1 µL genomic DNA. The following PCR profile was used: initial denaturation at 94 ^oC for 5 min; 40 cycles of 94 ^oC for 45 s for denaturation, annealing at 53.5 ^oC for 40s, and polymerization at 72 ^oC for 60 s; final elongation at 72 ^oC for 5 min. The SNP C1032T was detected by digesting 10 µL of the 527 bp PCR product with 15 u Eco72 I enzyme at 37 ^oC overnight. Restriction patterns were visualized by electrophoresis of the digestion product in a 1.5% agarose gel stained with ethidium bromide.

The PCR-RFLP method was developed successfully for genotyping the C1032T SNP in intron 2 of the chicken IGFBP2 gene, and 23 white and 25 black color chicken feathers were screened. Three genotypes were detected and defined as CC, CT, and TT. Digestion of the PCR product of C1032T gave rise to 3 restricted patterns named CC (50/477 bp), CT (50/477/527 bp) and TT(527 bp) (Figure 1).

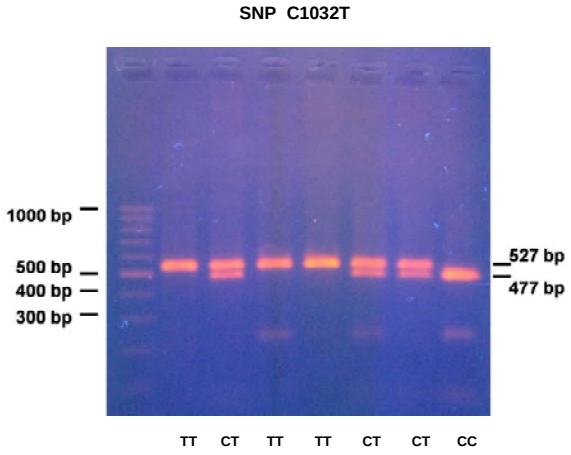

Figure 1. Gel picture of the chicken IGFBP 2 gene C1032T genotypes

Statistical Analysis

The association between the polymorphism and phenotypic trait was analyzed by factorial model of variance analysis. The model was used for native chicken population analysis, which fitted with genotyped and color feather as fixed effects. To investigate if there are differences among the color feather in IGFBP2 allele frequencies, χ^2 test for independence on allele frequencies for the color feather were performed using Suryo model (2005).

RESULTS AND DISCUSSION

The IGFBP2 polymorphism was significantly associated with body weight at hatch, 1,2, and 3 week of age (Table 1) for total black-white color feather, but not for either black or white color feather. The smallest body weight at hatch, one and two week of age was genotype CC, the medium body weight was TT, and the heaviest body weight was CT ($P \leq 0.05$). At the age of three week, body weight of the genotype CC was the smallest, but the body weight of genotype CC and CT was not different anymore. Starting at four week old until 12 week old, the body weight of genotype CC, CT, and TT, were not different, so the chicken with genotype CC, at the early life has slow growing chicken but at the end of the research can reach the same body weight with chicken genotype either CT or TT. The result agreed with the result of Lei et al. (2005) that SNP C1032T was associated with chicken body weight at hatch, 7, 14, 21, and 28 day of age. The result of the current study slightly different with the result of Li et al. (2003) experiment, that SNP C1032T was associated with weekly body weight from one week until 12 week of age. Leng, et al. (2009), shown that a C/A polymorphism was found in the 3′-flanking region of the IGFBP2 gene, and A allele associated with low body weight. In the study C/T polymorphism of IGFBP2, showed that C allele associated with low body weight at hatch, 7, 14, and 21 day of age.

Allele and genotype frequencies observed in the analyzed samples are given in Table 2. For all color feather and the total color, allele T was the most frequent allele and range from 0.52 (black color feather), 0.59 (white color feather), and 0.55 (total black-white color feathers). The frequency of CC homozygous genotype was the lowest (0.15 for the average black and white color feather), whereas CT genotype had the highest frequency (0.60 for average black and white color feather). The probability of random population was estimated by Chi-square (χ^2) test to examine Hardy-Weinberg equilibrium (HWE) at each color feather. The χ^2 test showed that χ^2 value for black (0.12) and total black-white color feather (3.2) were lower than χ^2 Table value (5.99), whereas for white color (6.7) was higher than χ^2 Table value at α 0.05. According to Suryo (2005), if χ^2 value lowers than χ^2 value Table ($\chi^2 \leq \chi^2$Table) so null hypothesis can be accepted, at the level α value 0.05. For white color

feather $\chi^2 \leq$ Table at the level α value 0.01 (9.21). according to χ^2-test, the genotype frequencies among black, white and total black-white colors feather did not differ from the expected Hardy-Weinberg equilibrium.

Table 1. Effect of IGFBP2 polymorphism on black and white Kampung chicken body weight

Age/ wks	Black			White			Total Black-White		
	CC	CT	TT	CC	CT	TT	CC	CT	TT
DOC	24.30	30.67	27.67	26.00	30.35	25.20	24.57[a]	30.48[c]	26.50[b]
1	38.30	43.83	43.00	36.00	45.06	38.80	38.02[a]	44.55[c]	41.00[b]
2	56.00	77.67	73.43	36.00	70.47	64.40	53.14[a]	73.45[c]	69.67[b]
3	92.50	128.33	116.57	60.00	114.00	118.40	87.85[a]	119.93[b]	117.33[b]
4	159.33	201.25	192.14	172.00	180.12	160.40	161.14	188.86	178.92
5	264.33	307.17	243.71	230.00	308.53	295.20	259.43	307.97	265.17
6	373.67	352.83	336.86	314.00	351.94	336.40	373.67	352.30	336.67
7	371.67	353.33	378.00	332.00	356.78	345.40	371.67	355.40	364.42
8	467.00	487.83	482.00	384.00	481.56	418.00	467.00	484.07	455.33
9	552.00	515.83	568.57	422.00	494.00	493.60	552.00	502.73	537.33
10	477.67	585.25	511.43	422.00	492.11	473.60	477.67	529.37	495.67
11	620.83	641.67	650.00	600.00	656.94	596.00	620.83	650.83	627.50
12	764.33	760.83	762.86	740.00	832.78	740.00	764.33	804.00	753.33

[a,b,c] Means within a row with no common superscript are different (P≤0. 05)

Table 2. Allele and genotype frequency of IGFBP2 gene in black, white, total black-white color feather

	n	Genotypes frequencies			Alleles frequencies		χ^2	P
		CC	CT	TT	C	T		
Black	25	0.24	0.48	0.28	0.48	0.52	0.12	NS
White	23	0.04	0.74	0.22	0.41	0.59	6.7	P≤0.05
Black-white	48	0.15	0.60	0.25	0.45	0.55	3.2	NS

CONCLUSIONS

The polymorphism of IGFBP2 gene was associated with body weight at hatch, body weight at 7, 14, and 21 day of age. A C/T polymorphism of IGFBP2, showed that C allele associated with low body weight at hatch, 7, 14, and 21 day of age. The frequency of restriction enzyme C/T alleles in the black color was 0.48 (C) and 0, 52 (T), for the white color 0.41 (C) and 0.59 (T), and for black and white 0.45 (C) and 0.55 (T). Genotype among black, white, and black-white colors feather did not differ from the expected Hardy-Weinberg equilibrium

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