

A Polymorphism of Insulin-Like Growth Factor Binding Protein 2 Gene Associated with Growth and Body Composition Traits in Kampung Chickens

(KERAGAMAN GEN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 2 YANG BERASOSIASI DENGAN SIFAT PERTUMBUHAN DAN KOMPOSISI TUBUH PADA AYAM KAMPUNG)

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Abstract

Insuline-like growth factor binding protein 2 (IGFBP2) is one of the principal binding proteins that has biological functions involved in growth, development, and differentiation. Selection for rapid growth based on molecular Marker Assisted Selection is required to increase production performance. The present study was designed to analyze the associations of IGFBP2 gene polymorphisms with chicken growth and body composition traits. Kampung chicken, a native chicken in Indonesia, is slow-growing chicken. A total of 59 males were used in the current study. Growth and body composition were measured in 24 wk of age. Primers for intron 2 region were designed from genomic chicken sequence. A c.1032C>T SNP of the IGFBP2 gene intron 2 region was detected and PCR-RFLP method was then used to genotype Kampung chicken population. The result showed that IGFBP2 polymorphism was significantly associated with body, carcass, breast, breast muscle, pectoralis minor, leg, and wings weight in Kampung chicken population ($P < 0.05$). The research suggests that the IGFBP2 gene could be a candidate gene that affects growth and body composition traits in chicken.

Keywords: kampung chicken; growth; body composition; insuline-like growth factor binding protein 2 gene; polymorphism

Abstrak

Insuline-like growth factor binding protein 2 (IGFBP2) merupakan protein pengikat utama yang memiliki fungsi biologi terlibat dalam pertumbuhan, perkembangan, dan diferensiasi. Program seleksi untuk sifat pertumbuhan cepat berdasarkan penanda molekuler sangat diperlukan untuk meningkatkan performa produksi. Terkait hal tersebut, penelitian ini didesain untuk menganalisis asosiasi keragaman gen IGFBP2 dengan sifat pertumbuhan dan komposisi tubuh pada ayam kampung. Ayam Kampung merupakan ayam asli Indonesia dan tergolong ayam dengan pertumbuhan lambat. Sebanyak 59 ayam Kampung jantan digunakan dalam penelitian ini. Pertumbuhan dan komposisi tubuh diukur pada umur 24 minggu. Sepasang primer didesain pada daerah intron 2 berdasarkan sekuen genom ayam. Sebuah SNP c.1032C>T pada gen IGFBP2 intron 2 dideteksi dan metode PCR-RFLP digunakan dalam menentukan genotype ayam Kampung. Hasil menunjukkan bahwa keragaman IGFBP2 signifikan berasosiasi dengan bobot badan, karkas, dada, otot dada, *pectoralis minor*, paha, dan sayap pada populasi ayam Kampung ($P < 0.05$). Penelitian ini menyarankan gen IGFBP2 dapat dijadikan kandidat gen yang mempengaruhi sifat pertumbuhan dan komposisi tubuh pada ayam.

Kata-kata kunci: ayam kampung; pertumbuhan; komposisi tubuh; *insuline-like growth factor binding protein 2*; keragaman

INTRODUCTION

Kampong chicken, a native chicken and one of genetic resources in Indonesia, has important roles in supplying egg and meat. Kampong chicken has no specific characteristics and usually raised free range in most rural area of Java, Indonesia (Nataamijaya, 2010; Riztyan *et al.*, 2011). The selection for rapid growth in meat-type chicken is required to increase production performance. This selection results in an increase of not only production performance but also physiological disorders (Deeb and Lamont, 2002). There is a negative correlation between production performance and fitness traits in the chickens (Martin *et al.*, 1990). Selection in Kampong chickens usually is based on their phenotypic. It is difficult to increase the production performance and fitness traits simultaneously. Selection efficiency in production performance can be increased by using molecular marked-assisted selection (Li *et al.*, 2003).

Insuline-like growth factors (IGF) are polypeptide that play an important role in cell growth and differentiation (Rechler, 1993). IGF-binding proteins extend the half-life of the IGFs and have shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They change the interaction of IGFs with their cell surface receptors (Chesik *et al.*, 2007).

There are 7 IGFBP that have been identified successfully, and they were named IGFBP1 to IGFBP7 (Shimasaki and Ling, 1991; Oh *et al.*, 1996). In mammals, these IGFBPs play a role in modulation of IGFs action by preventing insulin-like effects, regulation of halflife of IGFs in circulation, association with IGFs as carrier proteins and redistribution of IGFs between tissues and extracellular fluids (Kim *et al.*, 2010). Insulin-like growth factor binding protein 2 (IGFBP2) is one of 7 IGFBP in serum of different species and binds IGF (Drop *et al.*, 1992).

Previous studies have shown that some Single Nucleotide Polymorphisms (SNP) of IGFBP2 significantly affected growth traits (Lei *et al.*, 2005; Sri-Sudaryati *et al.*, 2013; Zhao *et al.*, 2015). Leng *et al.* (2009) reported that a polymorphism of IGFBP2 in the 3'-flanking region was associated with abdominal fat. In addition, IGFBP2 level negatively affected bone size and mineral content in mice, which suggested that it was an important regulator of bone biology *in vivo* (Eckstein *et al.*, 2002). Chicken IGFBP2 is highly expressed in many

tissues of embryos, such as eye, muscle, brain, liver, heart, kidney, ovary and intestine (Schoen *et al.*, 1995). Recently, IGFBP2 are detected by real-time Polymorphism Chain Reaction (rtPCR) technique in broiler chicken (Leach *et al.*, 2006; Lu *et al.*, 2010).

The objectives of the current study were to identify SNP in IGFBP2 gene intron 2 in Indonesian local chickens and to evaluate the associations between IGFBP2 SNP and traits of growth and body composition in Kampong chickens.

RESEARCH METHODS

Experimental Populations and Management

A total of 83 Kampong, 20 Merawang, 30 Sentul, 19 Pelung, and 9 broiler chickens were used in the current study to identify a polymorphism in IGFBP2 gene intron 2. For association study, there were only 69 Kampong chickens used in this study. All chickens had access to feed and water *ad libitum*. From hatch to 8 weeks of age, Kampong chickens received a starter feed (4,080 kcal of gross energy/kg and 19,03% of crude protein) and from 9 to 24 weeks of age, Kampong chickens were fed a grower diet (4,001 kcal of gross energy/kg and 17,42% of crude protein).

Phenotypic Measurements

Body weight and body composition traits were recorded at 24 weeks of age. These measurements included body weight (BW), carcass weight (CW), breast weight (BrW), breast muscle weight (BrMW), Pectoralis major weight (PmaW), Pectoralis minor weight (Pmiw), leg weight (LW), and wings weight (WW). All the traits were also expressed as a percentage of BW at 24 weeks of age.

DNA Isolation and PCR Amplification

Blood samples were collected from axillary vein. Genomic DNA were isolated according to Sambrook *et al.* (1989). The PCR primers of IGFBP2 gene intron 2 used in this study were based on previous study (Li *et al.*, 2006). This primers (52 GTC CCA GAT AAA CCT TGC T 32; 52 GCT GGC AAG GGG TCT G 32) were designed to amplify a 367-bp fragment by Primer 5.0 (Premier Biosoft International, Palo Alto, CA) according to the chicken genomic sequence in the GenBank database (accession number AY

326194). The PCR reaction conditions were 94°C for 5 min, 35 cycles of 94°C for 10 s, 60°C for 20 s, 72°C for 30 s, and an extension at 72°C for 5 min. The 25-µL reaction volume included 50 ng of DNA template, 1× reaction buffer, 5 pmol of each primer, 0.16 mM of deoxyribonucleotide triphosphate, 1.5 mM of MgCl₂, and 1 U of *Taq* polymerase.

Screening of the Population for Restriction Enzyme-Detectable SNP

A single nucleotide polymorphism (SNP) of the IGFBP2 gene was detected by digesting 7 µL of the 367 bp PCR product with 3 U of the *Eco72 I* enzyme (Thermo Fisher Scientific Inc., Lithuania) at 37°C overnight. The restriction digests were electrophoresed for 45 m at 100 V on a 2.0% agarose gel with ethidium bromide. Individual PCR-RFLP fragment sizes for the gene were determined by visualizing the band pattern under UV Transilluminator (Alpha-Imager® EP).

Statistical Analysis

The association between the polymorphism and the growth and body composition traits was analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC). The model was fitted with the genotype of IGFBP2 gene intron 2 (**G**: 3 levels: CC, CT, TT) as fixed effects, as follows, $Y = \mu + G + e$; Where *Y* is the dependent variable for trait measured in the population, μ is the overall population mean for traits, *G* is the fixed effects,

and *e* is the random error. Significant differences between means of the different genotypes were calculated using the Duncan test. Significance was determined as *P* < 0.05, unless otherwise specified.

RESULTS AND DISCUSSION

Polymorphism in the Intron 2 Region of Chicken IGFBP2 Gene

The present study identified a C/T SNP at base 1032 (accession number AY 326194) in the IGFBP2 gene intron 2 region. The polymorphism was associated with body composition traits of growing chickens. The examined SNP in intron region may either be causal or linked with functional polymorphisms of any region of the IGFBP2 gene or other nearby genes (Li *et al.*, 2006). Introns play an important role in transcriptional regulation and genom structure (Chorev and Carmel, 2012). In addition, a C/A polymorphism was found in the 32 -flanking region of the IGFBP2 gene at base 1196 (acesion number U15086), this SNP associated with fatness traits (Leng *et al.*, 2009).

A 367 bp in length was successfully amplified by using PCR. There was a C/T SNP at base 1032 (accession number AY326194). The PCR-RFLP method was used successfully for genotyping the SNP in intron 2 of the chicken IGFBP2 gene. Three genotypes were detected and defined as CC, CT, and TT (Figure 1).

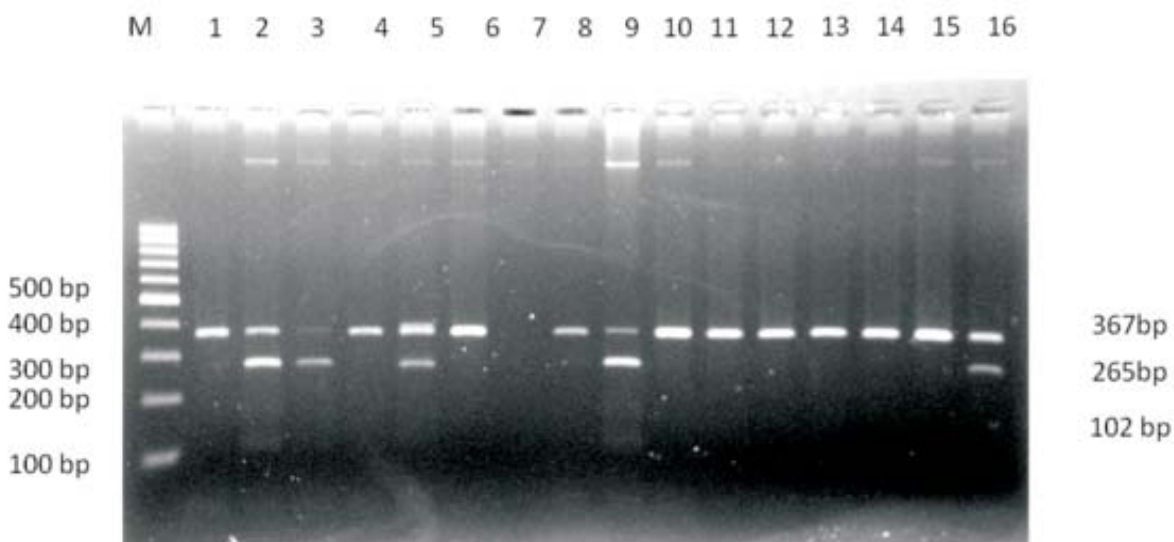


Figure 1. The PCR-RFLP pattern for IGFBP2 gene intron 2 region with *Eco72 I* restriction enzyme. M = markers. No.1,4,6,10-15 = CC genotype samples. No.3 = TT genotype samples. No. 2,5,9,16 = CT genotype samples.

The 367 bp fragment was digested with Eco72 I restriction enzyme. The digested PCR products had fragment sizes of 367 bp for the CC genotype, 265 bp and 102 bp for the TT genotype, and a combination of 367, 265 and 102 bp for CT genotype. These genotypes were named according to a mutation of nitrogen base from C (cytosine) to T (thymine). The Kampong chicken population was screened by PCR-RFLP.

The study found the c.1032C>T SNP in the intron 2 of IGFBP2 gene. The allele C frequency of Kampong, Merawang and Sentul was lower than the allele T frequency. On the other hand, the allele C frequency of Pelung was higher than the allele T frequency. The allele C frequency of Pelung was the highest among five distinct populations. The allele T frequency of Sentul was the highest among five distinct populations (Table 1). On the other hand, the allele T frequency of Sentul was the highest among five distinct populations. Pelung is one of local chickens that has bigger skeletal traits than the others.

IGFBP2 is one of the principal binding proteins secreted by differentiating white preadipocytes. IGFBP2 has a potential role in the development of obesity. Wheatcroft *et al.* (2007) reported that an overexpression of IGFBP2 was associated with the reduction of susceptibility to obesity and improvement of insulin sensitivity in rat. Chicken IGFBP2 gene is highly expressed in many tissues of embryos and consists of four exons and spans more than 38 kb on chromosome 7 (GGA7) (Schoen *et al.*, 1995).

Li *et al.* (2006) reported that allele A was associated with high skeletal measurements. The result of chi-square (X^2) test showed that the distribution of genotypes derived from second intron of IGFBP2 gene in Kampong, merawang, pelung and broiler population was in Hardy-Weinberg equilibrium. The population of sentul

chicken was not in Hardy-Weinberg equilibrium. A certain population was in Hardy-Weinberg equilibrium if the genotype frequencies and allele frequencies were constant from one generation to the next generation resulted from gametes fussion randomly in large population size (Allendorf *et al.*, 2007).

Association of IGFBP2 Gene SNP with Growth and Body Composition

The IGFBP2 polymorphism was generally significantly associated with body composition. There were significant associations between the IGFBP2 polymorphism and BW, CW, BrW, BrMW, PmiW, LW and WW. The PmaW was not significantly different among three genotypes. Kampong Chicken with the IGFBP2-TT genotype had higher BW, CW, BrW, LW, and WW than two another genotypes. Kampong Chicken with the IGFBP2-CC genotype had higher BrMW and PMiW than two another genotypes. Kampong Chicken with the IGFBP2-CT genotype was the lowest in all parameters. This might indicate that there are negative effects of heterosis (Table 2).

The study of the candidate gene is one of primary methods to find QTL responsible for genetic variation intraits of interest and to determine whether specific genes are related to economic traits in farm animals (Rothschild and Soller, 1997; Li *et al.*, 2003). A previous study has shown that a QTL for carcass weight was mapped (about 2.3 to 29 Mb) on GGA7 (De Koning *et al.*, 2003), which cover the chicken IGFBP2 gene (23 to 24 Mb). In addition, Ikeobi *et al.* (2002) showed that the QTL for fat deposition was mapped between marker brackets LEI0064 and ROS0019 (75 kb to 27 Mb) on GGA7 in the chicken linkage map, which cover the chicken IGFBP2 gene (23 to 24 Mb).

Li *et al.* (2006) reported that the c.1032C>T SNP associated with growth, skeletal measu-

Table 1. The frequency of alleles in kampong chicken population

Line	n	CC	CT	TT	C	T	X ²
Kampong	83	0.337 (28) ¹	0.386 (32)	0.277 (23)	0.530	0.470	4.24
Merawang	20	0.150 (3)	0.450 (9)	0.400 (8)	0.375	0.625	0.03
Sentul	30	0.200 (6)	0.167 (5)	0.633 (19)	0.283	0.717	10.44*
Pelung	19	0.474 (9)	0.474 (9)	0.052 (1)	0.711	0.289	0.44
Broiler	9	0.778 (7)	0.222 (2)	0.000 (0)	0.889	0.111	0.14

*Means within a row with no common superscript are different $X^2_{0.05(1)} = 3.84$.

¹ Numbers shown in parentheses are the number of individuals with the specified genotype.

Table 2. Effect of The IGFBP2 genotype on growth and body composition traits in Kampong chicken (Means±SE)

Traits ¹	Genotype		
	CC (22) ²	CT (23)	TT (14)
BW ³	1697.41 ± 225.39 ^{ab}	1585.30 ± 161.91 ^a	1740.07 ± 213.43 ^b
CW	1096.09 ± 205.22 ^{ab}	1017.39 ± 157.07 ^a	1151.36 ± 220.94 ^b
BrW	274.41 ± 40.18 ^{ab}	251.70 ± 37.22 ^a	277.43 ± 33.50 ^b
BrMW	195.63 ± 29.46 ^a	172.00 ± 30.53 ^b	189.69 ± 34.79 ^{ab}
PmaW	136.63 ± 19.15	123.10 ± 21.23	134.31 ± 25.01
PmiW	59.01 ± 11.82 ^a	48.90 ± 11.50 ^b	55.37 ± 13.17 ^{ab}
LW	400.27 ± 69.92 ^{ab}	381.39 ± 60.78 ^a	430.14 ± 69.95 ^b
WW	139.64 ± 22.05 ^{ab}	128.39 ± 17.50 ^a	146.86 ± 25.12 ^b

a,b Means within a row with no common superscript are different ($P < 0.05$).

1 BW = body weight, CW = carcass weight, BrW = breast weight, BrMW = breast muscle weight, PmaW = pectoralis major weight, PmiW = pectoralis minor weight, LW = leg weight, WW = wings weight.

2 Numbers shown in parentheses are the number of individuals with the specified genotype.

3 All of traits are in grams.

rements and body compositions traits in the F2 population of NEAU chickens. In the previous study, the homozygous chickens for the B allele were the highest for growth traits and the lowest for skeletal measurement traits. On the other hand, the homozygous chickens for the A allele were the lowest for the body composition traits.

CONCLUSION

The population of Kampong chicken based on IGFBP2 gene polymorphism was polymorphic. Three genotypes (CC, CT, and TT) were found in this study. On average, Kampong chickens with the IGFBP2-CT genotype grew lower than the others.

SUGGESTION

All of these results showed that the c.1032C>T SNP of IGFBP2 intron 2 region could be possible as a candidate gene useful for altering growth rate and body composition.

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