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The Effect of Insulin like Growth Factor Binding Protein 2 Gene on Kampung Chicken Growth Rate

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Abstract: Insulin-like growth factor binding protein 2 regulates a broad spectrum of biological activities involved in growth, development and differentiation. The current study was designed to investigate the associations of IGFBP2 gene polymorphisms with the variance components and genetic parameters of four weekly intervals of growth rate (0-4, 4-8, 8-12 weeks) of the Kampung chicken that were evaluated with classical models of quantitative genetics. Thirty two females and 16 males Kampung chicken were genotyped with IGFBP2 gene using PCR-RFLP method. Retrieved 3 genotype (CC, CT and TT) and 2 alleles (C and T). The influence of average effect of the C allele was greater than T allele on a 4-8 weeks intervals of growth rate, meanwhile for the males turn into 0-4 and 8-12 weeks interval growth rate. Ratio component additive and dominant deviation to each genotype is always equal for each interval growth rate. Variance component from heterozygote nearly zero when frequency of homozygote nearly equal and ratio variance dominance were opposite to allele frequency. Narrow-sense heritability based on genetic variance component of 0-4, 4-8 and 8-12 weeks interval growth rate for female were 0.01, 0.47 and 0.87 while for male chicken were 0.94, 0.07 and 0.12, respectively. It was concluded that IGFBP 2 gene association with growth rate on 4-8 and 8-12 weeks on females but in males only effect on growth rate on 0-4 weeks.

Key words: Kampung chicken, growth rate, heritability

INTRODUCTION

Kampung chickens are generally of small body size, having slow growth rate with different colors of plumage and of dual purpose type with variable body conformation and physical characteristics. Body weight is variable and they lack uniformity in growth. According to Nataamijaya (2010), Kampung chicken is a local ecotype of Indonesian native chicken which has no specific characteristics, usually raised free range in most rural areas of Java, Indonesia (Riztyan *et al*., 2011). The genes of somatotropic axis play a central role in the regulation of growth and development (Kim, 2010). Insulin-like growth factor binding protein 2 regulates a broad spectrum of biological activities involved in growth, development and differentiation (Li *et al*., 2006; Lei *et al*., 2005 and 2007). Previous studies have shown that some Single Nucleotide Polymorphisms (SNP) of the somatotropic axis genes indeed affected growth traits significantly (Amills *et al*., 2003; Lei *et al*., 2005 and 2007; Nie *et al*., 2005b; Li *et al*., 2006; Sri-Sudaryati *et al*., 2010).

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. SNP are a new type of DNA polymorphism, mostly bi-allelic but widely distributed along the chicken genome (Vignal *et al*., 2002). The chicken IGFBP 2 gene spans approximately 38 kb and is located on chromosome 7. 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).

For such genetic improvement, knowledge of heritability is essential for planning, efficient breeding programs and for predicting response selection (Dana *et al*., 2011). Narrow-sense h^2 is most important in animal and plant selection programs. Narrow-sense heritability, $h^2 = V_A/V_P$, captures only that proportion of genetic variation that is due to additive genetic values (V_A) (Toghiani, 2012). Genotype conferring a certain value on the individual and the environment causing a deviation from this, in one direction or the other, symbolically P = G+E, where P is the phenotypic value, G is the genotypic value and E is the environmental deviation. The mean environmental deviation in the population as a whole is taken to be zero, so that the mean phenotypic value is equal to the mean genotypic value (Falconer and MacKay, 1997).

Heritability estimates reported in the literature for body weight in native chickens vary considerably, depend on the region and age. Body weight heritability of Iranian chicken at one day old, 8 and 12 weeks was 0.56, 0.44 and 0.51, respectively Bahmanimehr (2012). According to Dana *et al*. (2011), heritability of body weight of

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Ethiopian chicken was low on hatch time (0.15±0.08) and become moderate at 6 weeks2 old (0.40±0.23). Pedro-Gonzales *et al.* (2003), reported that heritability of Mexican native chicken were 0, 15 at hatch body weight, 0.20 at 4 weeks, 0.21 at 8 weeks and 0, 13 at 12 weeks body weight.

The purpose of the present study was to observe the effect of the growth rate-correlated genes of variance component and parameter genetic on four week interval growth rate of Kampung chicken.

MATERIALS AND METHODS

A total of 48 samples (32 females and 16 males) Kampung chicken were used in this study. All of the chickens were raised in rice hull litter cage. During 0-6 weeks of age, the chicken were fed by commercial broiler feed contain 21% CP and ME 3.200 kcal/kg and then changed to Kampung feed until the bird reach 12 weeks of age. Kampung chicken contain 13% CP and 2.150 kcal/kg ME.

Genomic DNA was isolated from venous blood using EDTA. The SNP C1032T (GenBank accession number AY 326194) was used for genotyping. PCR-RFLP method using Eco 72 I restriction enzyme was used for the digesting of PCR product (527 bp). The SNP C1032T was detected by digesting 10 μL of the 527 bp PCR product with 15 u Eco72 I enzyme at 37°C overnight. Restriction patterns were visualized by electrophoresis of the digestion product in a 1.5% agarose gel stained with good view.

Body weights of the progeny were taken at day 0 (hatching) and at the end of every week. Birds were individually weighed in order to determine their Relative Growth (RG) as:

$$RG = 100 \times \frac{G2 - G1}{G1}$$

(De Smit, 2005). G_1 is outset body weight and G_2 is the latest body weight. Relative growth were taken 0-4, 4-8 and 8-12 weeks interval.

Chi-square test (x^2) according Kaps and Lamberson (2004) was used to examine the Hardy-Weinberg equilibrium:

$$\chi^2 = \sum \frac{\left[Y_1 - E(Y_1)\right]^2}{E(Y_1)}$$

Where:

y_1 = No. of observation
Ey_1 = Expected number of observation

Pirchner (1979), Falconer and MacKay (1997), analyzed that deviation from point origin (o) is the average of homozygous phenotype.

The population mean value as expressed as a deviation from the point origin (m):

$$m = a (p-q)+2pqd$$

Where:

a = Deviation of homozygous dominance
p = Frequency allele C and q = frequency allele T
d = Genotypic value of heterozygote

Based on relative growth and genotypes of the chickens, variance genetic components were estimated. Average value effect alleles were analyzing by Griffiths *et al.* (2000). The formula of contribution value allele:

$$C = \frac{2p^2RG_{CC} + 2pqRG_{CT}}{2p^2 + 2pq}$$

and allele:

$$T = \frac{2p^2RG_{TT} + 2pqRG_{CT}}{2q^2 + 2pq}$$

Where:

p = Frequency allele C and q = frequency allele T
RG_{CC} = Relative growth of genotype CC
RG_{CT} = Relative growth of genotype CT
RG_{TT} = Relative growth of genotype TT

Average effect alleles were estimated by Pirchner (1979); Falconer and MacKay (1997).

Average effect allele:

$$C = q [a+d (q-p)].$$

Average effect allele:

$$T = -p [a + d (q-p)]$$

Where:

p = Frequency allele C
q = Frequency allele of T
a = Deviation of homozygous dominant and
d = The genotypic value of heterozygote

Variance component genetic (V_G) were estimated by Pirchner (1979); Falconer and MacKay (1997):

$$V_G = V_A + V_D$$

Variance component additive (V_A) = $2pq [a+d (q-p)]^2$ and variance component dominance (V_D) = $4p^2q^2d^2$.

Variance additive components were estimated by Pirchner (1979).

$$\text{Variance genotype CC} = p^2 (A_{CC})^2$$

$$\text{Variance genotype CT} = 2pq (A_{CT})^2$$

$$\text{Variance genotype TT} = q^2 (A_{TT})^2$$

where, A_{CC}, A_{CT} and A_{TT} were additive value of genotype CC, CT and TT, respectively.

Narrow-sense heritability were estimated by Pirchner (1979), Falconer and MacKay (1997) and Templeton (2006):

$$h^2 = \frac{V_A}{V_P}$$

if $V_P = V_G$, so:

$$h^2 = \frac{V_A}{V_G}$$

RESULTS AND DISCUSSION

SNP genotypes of the chicken IGFBP2: The PCR-RFLP method was developed successfully for genotyping the C1032T SNP in intron 2 of the chicken IGFBP2 gene. Digestion of the PCR product of C1032T gave rise to 3 restricted patterns named CC (477 bp), CT (477/527 bp) and TT (527 bp) (Fig. 1):

Hardy-Weinberg equilibrium: The probability of random population was estimated by Chi-square (x^2) test to examine Hardy-Weinberg Equilibrium (HWE) at each mating and sex. Alleles and genotypes frequencies observed in the analyzed samples are given in Table 1. The x^2 test showed that x^2 value for all mating either females or males were lower than Table value of x^2 (5.99) at α 0.05. According to Kaps and Lamberson (2004), if calculated value of x^2 lowers than Table value of x^2 ($x^2_c \leq x^2_t$) so null hypothesis can be accepted, at the level α value 0.05. According to x^2-test, the genotype frequencies of females and males Kampung chicken did not differ from the expected Hardy-Weinberg equilibrium.

Association of SNP of the IGFBP2 gene with four weekly growth rate: Two ways analyzed variance according Kaps and Lamberson (2004) was used to analyzed genotype and sex on four weeks interval growth rate (Table 2). There were no significant difference between CC, CT and TT genotype and sex on growth rate of Kampung chicken. Lack of uniformity on growth rate as a source of the no differences. A high standard deviation indicated the wide variability of body weight and uniformity of growth rate of Kampung chicken.

The effect of the IGFBP2 gene with four weekly growth rate: Deviation of the point origin (m) female and male showed were not the same value. 0n 0-4 weeks interval growth rate, was positive value on female and negative value on male chicken and on 4-8 weeks interval growth rate become opposite. The deviation of the point origin tend to decrease with the age of the chicken (Table 3). Population average value (m+o), female were lower than male and the value tend to decrease with the age of the chicken. The population average value and allele contribution value male higher than female for all the four weeks interval growth rate. similar to body weight of male higher than female body weight. Allele contribution value depend on sex and interval growth rate. Allele contribution value of allele T higher than allele C during 0-4 weeks interval growth rate for both female and on the contrary allele C higher than allele T for male chicken. On 4-8 weeks interval growth rate, change to be opposite, allele contribution of allele C higher than allele T for female chicken and on the contrary, allele contribution allele T higher than allele C for male chicken. On 8-12 weeks interval growth rate, for both female and male, were changed back to be like on 0-4 weeks interval growth rate. The role of allele changed by interval growth rate and sex of the chicken. The influence of allele C and T on growth rate were opposite between female and male chicken.

Animal with a rare allele will have a larger breeding value either positive or negative value: (Falconer and MacKay, 1979). Allele frequency of allele C either females or males chicken were lower than allele T, so average allele effect of C allele either positive or negative values always higher than T allele. An average allele effect were associated positively with allele contribution value. Although breeding value C allele positive or negative is greater than T allele, however if the allele contribution value T allele is larger than the C allele, causing average allele effect C allele will worth negative as shown at Table 3.

An individuals breeding value or additive value can also be described as the sum of the average effect of the individuals alleles. It is showed in Table 4, that additive value of each genotype were the sum of the average effect of the individual alleles. On 0-4 weeks interval growth rate for female chicken showed that allele contribution value of allele T higher than allele C but allele frequency of C allele lower than T allele, so additive value of CC genotype higher was higher than TT genotype but in negative value. All of additive value of CC genotype either females or males chicken were higher than TT genotype but when allele contribution of C allele was lower than T allele caused additive value of CC genotype worth negatively.

Total additive value depend on allele contribution value and allele frequencies. When allele contribution value of C allele lower than T allele and the allele frequency of C allele lower than T allele, the total additive value become negative (females on 0-4 and 8-12 weeks and males on 4-8 weeks) but when allele contribution of C allele higher than T allele and the frequency of C allele lower than T allele, the total additive value become positive (females on 4-8 weeks and males 0-4 and 8-12 weeks). Ratio each genotype to total additive value or dominance deviation were equal for each interval growth rate. Ratio CT genotype to total additive value nearly zero but for CC and TT genotype nearly five times for females and twice for males chicken. Ratio CC, CT and TT genotype to total dominance deviation nearly equal. Ratio variance dominance of CC, CT and TT genotype were opposite to allele frequency.

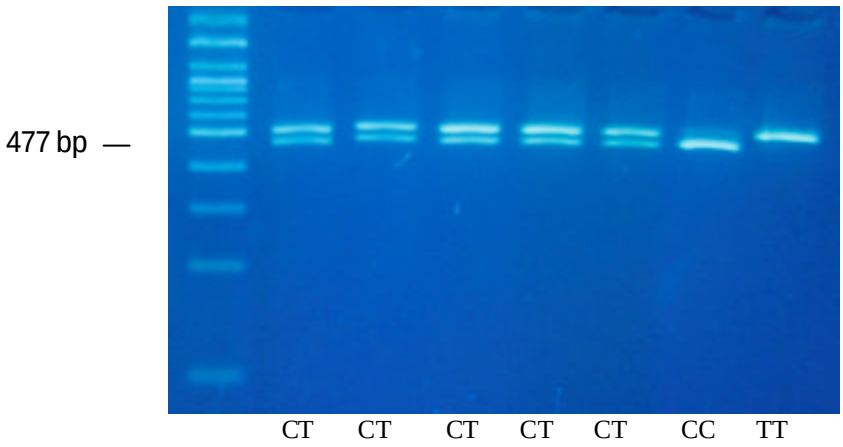

Fig. 1: Gel picture of the chicken IGFBP 2 gene C1032T genotypes

Table 1: Details of single nucleotide polymorphism and primer

Nucleotide constitutes	Sequence ID	SNP (bp)	Length	Temp (°C)	Enzyme
AFF 5'-TTTGGTTGAGTCCTAGGCTTG-3' AFR 5'-GGCGTACTACACTGCAGAGG–3'	AY 326194	C1032T	527 bp	53.5	*Eco*72 I

Table 2: Allele and genotype frequency of IGFBP2 gene of Kampung chicken

		Genotypes frequencies			Alleles frequencies			
Sex	n	CC	CT	TT	C	T	x^2	p-value
Female	32	15.62	62.50	21.88	0.47	0.53	2.08	NS
Male	16	12.50	56.25	31.25	0.41	0.59	0.44	NS

Table 3: Four weekly interval growth rate based upon sex and genotypes of Kampung chicken

			Four weekly interval growth rate		
Items	n	Genotypes	0-4	4-8	8-12
Female	5	CC	509.93±73.27	206.02±105.72	65.68±18.43
	20	CT	555.61±169.92	149.70±73.08	66.34±50.14
	7	TT	521.26±230.53	145.70±50.09	76.37±50.80
Male	2	CC	611.54±92.47	166.76±79.00	74.74±1.86
	9	CT	539.84±115.35	199.73±97.49	93.34±47.65
	5	TT	504.77±48.43	184.50±84.72	71.53±48.62

Table 4: Deviation of the point of origin (m), population average value (m+o), allele contribution value, and alleles effect of the female and male Kampung Chicken

				Allele contribution value		Average allele effect	
Four weekly growth rate	Sex	m	m+o	C	T	C	T
0-4	Female	20.28	535.87	534.14	537.40	-1.73	1.53
4-8	p = 0.47	-22.51	153.35	158.21	138.99	14.66	-13.00
8-12		1.72	72.75	71.12	74.88	-2.74	2.43
0-4	Male	-18.47	539.68	569.24	519.15	29.55	-20.54
4-8	p = 0.41	13.26	188.89	186.21	190.74	-2.67	1.86
8-12		9.49	82.62	85.71	80.47	3.09	-2.15

Genotypic value as the sum of the additive and dominance deviation value (Table 5). Genotypic value of each genotype depends on the value of each additive value and the dominance deviation value while the value of the additive value depends on the value of each allele and the value of each allele frequency depends on the allele frequency. In other words the value of the genotypic value and additive value depends on the average allele value and allele frequency.

The mean environmental deviation in the population as a whole is taken to be zero, so that the mean phenotypic value is equal to the mean genotypic value (Falconer and MacKay, 1997). Narrow sense of heritability is:

Table 5: Additive and dominance deviation value, variance additive and variance dominance deviation value of Kampung chicken

Four weekly growth rate	Female (p = 0.47)				Male (p = 0.41)			
	CC	CT	TT	Total	CC	CT	TT	Total
			Additive value (A)					
0-4	-3.46	-0.20	3.07	-0.59	59.10	9.02	-41.07	27.05
4-8	29.33	1.66	-26.01	4.98	-5.35	-0.82	3.72	-2.45
8-12	-5.49	-0.31	4.87	-0.93	6.19	0.94	-4.30	2.83
			Dominance deviation (D)					
0-4	-22.48	19.94	-17.68	-20.22	12.75	-8.86	6.16	10.05
4-8	23.35	-20.71	18.36	21.00	-16.78	11.66	-8.10	-13.22
8-12	-1.58	1.40	-1.24	-1.42	-14.07	9.78	-6.79	-11.08
			Variance additive value (V_A)					
0-4	2.64	0.02	2.64	5.31	587.22	39.33	587.22	1213.78
4-8	189.98	1.37	189.98	381.34	4.81	0.32	4.81	9.94
8-12	6.65	0.05	6.65	13.35	6.43	0.43	6.43	13.29
			Variance dominance deviation (V_D)					
0-4	111.64	198.00	87.79	397.42	27.33	37.98	13.20	78.51
4-8	120.42	213.58	94.70	428.70	47.32	65.77	22.85	135.95
8-12	0.55	0.98	0.43	1.97	33.26	46.23	16.06	95.55
			Ratio additive value to total					
	5.89	0.33	-5.22		2.19	0.33	-1.52	
			Ratio dominance deviation to total					
	1.11	-0.99	0.87		1.27	-0.88	0.61	
			Ratio variance additive value to total					
	0.50	0.00	0.50		0.48	0.03	0.48	
			Ratio variance dominance deviation to total					
	0.28	0.50	0.22		0.35	0.48	0.17	

Table 6: Variance additive, variance dominance, variance genotypic, and heritability of female and male Kampung chicken

Sex	Four weekly growth rate	$V_{(A)}$	$V_{(D)}$	$V_{(G)}$	h^2
Female, p = 0.47	0-4	5.31	397.42	402.73	0.01
	4-8	381.34	428.70	810.04	0.47
	8-12	13.35	1.97	15.31	0.87
Male, p = 0.41	0-4	1213.78	78.51	1292.29	0.94
	4-8	9.94	135.95	145.88	0.07
	8-12	13.29	95.55	108.85	0.12

$$\frac{V_{(A)}}{V_{(P)}}$$

(Templeton, 2006), if $V_{(P)}$ is equal to $V_{(G)}$, so narrow sense heritability is:

$$\frac{V_{(A)}}{V_{(G)}}$$

Heritability of female chicken was low during 0-4 weeks interval growth rate, then moderate and become high on the last growth rate. Meanwhile for male chicken, heritability was high on 0-4 weeks interval growth rate and then low during 4-8 and 8-12 weeks interval growth rate. Heritability of native chicken vary considerably. Heritability was high for Iranian chicken (Bahmanimehr, 2012) and low for Ethiopian chicken (Dana *et al*., 2011) and Mexican chicken (Pedro-Gonzales *et al*., 2003).

Conclusion: Average allele effect of C and T allele depend on allele contribution value and allele frequency. When allele contribution value of C allele higher then T allele and allele frequency C allele lower than T allele, average allele value of C allele become higher negative value. Average allele effect of C allele was higher than T allele on 4-8 weeks interval growth rate for females, meanwhile 0-4 and 8-12 weeks for males chicken. Ratio additive and dominance deviation to total were always equal in every stage of four weekly interval growth rate. Contribution of heterozygote genotype on variance additive nearly zero when frequency p and q nearly equal. Ratio dominance additive to total is apposite of allele frequency. Narrow-sense heritability of 0-4, 4-8, 8-12 were 0.01, 0.047 and 0, 87 for females and 0.94, 0.07 and 0.12 for males chicken, respectively. The effect of IGFBP2 gene were associated with growth rate on 4-8 and 8-12 weeks on females but in males only effect on growth rate on 0-4 weeks.

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