Polymorphisms of Growth Hormone (GH|MspI) Gene in Indonesia Local Chicken and the Crossbred using PCR-RFLP

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ABSTRACT

The aimed of the research was to identify the chicken growth hormone (cGH) gene polymorphisms in local chicken, the crossbred, commercial meat type and layer. cGH gene plays a crucial role in controlling growth and metabolism, leading to potential correlation between cGH gene polymorphisms and economic traits such as meat and egg production. Polymorphism in intron 4 of cGH gene was studied in 229 chicken blood samples consist of kampung, pelung, sentul, commercial meat type, layer, merawang, commercial meat type x kampung, kampung x commercial meat type, sentul x kampung and pelung x sentul chickens. A specific primer set was used to amplify a fragment of growth hormone locus using PCR. PCR products were digested with MspIrestriction endonucleases. The amplified fragment digested with MspIenzyme revealed 2 alleles A and B with the frequency of 0.88-0.96 and 0.04-0.12, except in layer chicken only had 1 allele A with the frequency of 1.00. Results showed that cGH gene in *Msp*I locus at kampung, pelung, sentul, commercial meat type, merawang, commercial meat type x kampung, kampung x commercial meat type, sentul x kampung and pelung x sentul chickens were polymorphics, except the layer chicken was monomorphics. The populations of Indonesia local chicken that was studied in this research had low heterozygosity but it was still in the Hardy-Weinberg equilibrium.

Key Words: cGH, Local chicken, MspI, Polymorphisms

INTRODUCTION

Indonesian local chicken has genetic potency, because there are adapted to the tropical environment and strong immunity, and high ecomonic value for meat and egg production. Local chicken, usually is used to produce meat and egg as final stock. There are limited program to to create superior breed from the local chicken breed. The intensive research in local chicken breeding and selection based on economic traits would improve the genetic quality.

Chicken growth hormone (cGH) gene plays a crucial role in controlling growth and metabolism. cGH gene was located in 1q4 chromosome with the length of 3901 bp consist of 5 exon and 4 intron (Tanaka et al., 1992). Kuhnlein et al. (1997) reported that there were alleles in intron 1, 3 and 4 of cGH gene assosiated with egg production and resistance to marek disease and avian leukosis. Makhsous et al. (2013) reported that there was 1 SacI locus in intron 4 of cGH gene assosiated with egg number and laying rate. Biangxue et al. (2003) reported that there was 1 MspI locus in intron 4 of cGH gene assosiated with breast muscle and abdominal fat rate. Therefore, cGH gene can be used as genetic marker through selection to enhance local chicken performance.

The objective of this research was identify the cGH gene polymorphisms in local chicken, the crossbred, commercial meat type and layer using PCR-RFLP.

MATERIALS AND METHODS

General: Blood samples were collected from 229 heads of local chiken, the crossbred, and commercial meat type and layer belonging to the Breeding and Gentic Laboratory, Faculty of

Animal Science, IPB. Genomic DNA was extracted from blood samples using phenol chloroform method (Sambrook et al., 1989).

A pair of primers was designed consist of forward 5'-GCCTGGGAGCAAA CAAACCC-3' and reverse5'-CCATGACACTTCAGCTGCAGC-3'. The primer pair was used to amplify 367 bp fragment of cGH gene containing 1 RFLP of T-109C. The PCR was performed in a final volume of 16 µl containing 2 µl extracted DNA, 7.5 µl GoTaq® Green Master Mix (Promega), 6.2 µl DW and 0.3 µl primer in a thermocycler with the following profile: initial denaturation of 5 min at 95ºC; 35 cycles of 94 ºC for 10 s, 60 ºC for 20 s and 72 ºC for 30 s with a final elongation of 5 min at 72 ºC.

The fragment amplified by primer pair was digested with *Msp*I restriction endonucleases, and then electrophoresed on 2% agarose gel for genotyping the polymorphisms of T-109C.

Statistic: Allelic frequency was calculated based on Nei and Kumar (2000) using the following formula:

$$x_i = \frac{2n_{ii} + \sum n_{ij}}{2N}$$

where: x_i = iallelic frquencies, n_{ii} = number of individual with ii genotype, n_{ij} = number of individual with ij genotype and N = number of total individual.

Observed (Ho) and expecetd (He) heterozigosity were calculated based on Weir (1996) and Nei and Kumar (2000) using the following formula:

$$Ho = \sum_{i \neq j} \frac{n_{ij}}{N}$$

$$He = 1 - \sum_{i=1}^{q} x_1^2$$

where: Ho = observed heterozigosity, n_{ij} = number of individual with ij genotype, N = number of total individual, He = expected heterozigosity, x_1 = allelic frequencies and q = number of allele

Hardy-weinberg equilibrium with chi-square test was calculated based on Hartl and Clark (1997) using the following formula:

$$X^2 = \sum \frac{(O - E)^2}{E}$$

where: X^2 = chi-square, O = number of observed genotype and E = number of expected genotype.

RESULTS AND DISCUSSION

Results of DNA visualization found that 1 MspI locus in 108 base of intron 4 or 3197 base of complete sequence of cGH gene. Genotyping results showed that there was mutation in 109 base.The mutation was transition mutation of T-109C. Research found that 2 alleles A and B with 3 genotypes AA, AB and BB. Allele A well marked with 1 DNA tape in 367 bp and allele B well marked with 2 DNA tape in 259 and 108 bp. The DNA visualization displayed in Figure 1.

The allele can be classified as polymorphics allele if the allelic frequency less than 1.00 (Nei 1987). The frequency of allele A in all populations was 0.88-0.96 except in layer and the frequency of allele B was 0.04-0.12 except in layer. It means that cGH gene in MspI locus was polymorphics except in layer because the allele frequency reached 1.00. Makhsous et al.

(2013) and Nei et al. (2002) also found A allele was dominant in Iran and China local chicken populations. Biangxue et al. (2003) reported that there was T-C mutation in commercial meat type crossing with silky population.

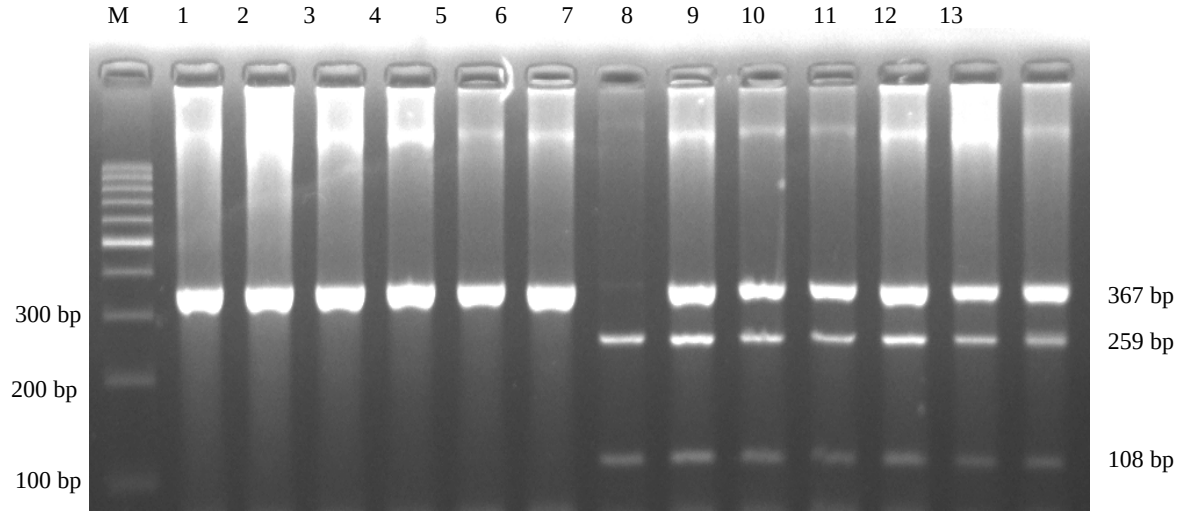

Figure 1. Visualization of cGH gene on 2% agarose gel. M was marker. I-9 were DNA sample of local chicken

This mutation assosiated with breast muscle and abdominal fate rate. Individual with BB genotype (mutation) had higher breast muscle rate (6.64%) and lower abdominal fat rate (1.98%) than individual with AB (6.59%; 2.66%) and BB (6.22%; 3.21%) genotype. The allelic frequency of chicken populations displayed in Table 1.

Table 1. Allelic frequency of chicken populations

Chicken populations	Alleles	
	A	B
Kampung	0.91	0.09
Pelung	0.90	0.10
Sentul	0.92	0.08
Commercial meat type	0.95	0.05
Layer	1.00	0.00
Merawang	0.96	0.04
Commercial meat type x Kampung	0.88	0.12
Kampung x Commercial meat type	0.95	0.05
Sentul x Kampung	0.96	0.04
Pelung x Sentul	0.95	0.05

Observed heterozigosity (Ho) in local chicken populations was 0.08-0.17, whereas Ho less than 0.5 show that the population classified as low heterozigosity. It means that Indonesia local chicken had low heterozigosity. Heterozigosity is influenced by some factors such as number of individual in population, number of allele and allele frequency (Allendorf and Luikart 2006). The heterozigosity of chicken populations displayed in Table 2.

The X^2 observed in local chicken populations was not different with X^2 table. It means that the local chicken populations was in hardy-weinberg equilibrium. Some factors that can influence the hardy-weinberg equilibrium was migration, mutation and genetic drift (Noor 2010). There was a mutation in this research but there was no effect on the population equilibrium. It could happened because of small number in mutation individual, so it did not give effect on population equilibrium.

Table 2. Heterozigosity of Chicken Populations

Chicken populations	n	Expected (He)	Observed (Ho)
Kampung	29	0.16	0.17
Pelung	5	0.18	0.20
Sentul	32	0.15	0.16
Commercial meat type	11	0.10	0.09
Layer	14	0.00	0.00
Merawang	23	0.07	0.09
Commercial meat type x Kampung	12	0.21	0.08
Kampung x Commercial meat type	33	0.10	0.09
Sentul x Kampung	50	0.15	0.08
Pelung x Sentul	20	0.10	0.10

The number of individual with BB genotype (mutation) less than 10%. The result of chi-square test for hardy-weinberg equilibrium displayed in Table 3.

Table 3. Hardy-Weinberg Equilibrium of Chicken Populations

Chicken populations	n	X^2 observed	X^2 table
Kampung	29	1.04^{tn}	3.84
Pelung	5	1.20^{tn}	3.84
Sentul	32	1.04^{tn}	3.84
Commercial meat type	11	1.50^{tn}	3.84
Layer	14	-	-
Merawang	23	1.04^{tn}	3.84
Commercial meat type x Kampung	12	1.33^{tn}	3.84
Kampung x Commercial meat type	33	1.25^{tn}	3.84
Sentul x Kampung	50	1.02^{tn}	3.84
Pelung x Sentul	20	1.05^{tn}	3.84

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