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Association of cGH *Eco*RV Gene with Production in Tolaki Chicken

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Abstract

cGH (Chicken Growth Hormone) gene plays a crucial role in production responses of chicken. The objective of the research was to investigate the association of cGH gene with production in Tolaki chicken. Tolaki chicken is native chickens from Southeast Sulawesi Province of Indonesian. cGH gene was genotyped in 58 Tolaki chicken with PCR-RFLP. PCR was used to amplify genomic DNA for GH gene (399 bp). The amplicon was cutted by *Eco*RV and produced three genotypes: AA, AG, and GG and two alleles: A and G allele. The study showed the association of GH gene polymorphism with Production traits. GG genotype have better production (daily weight gain and feed conversion) than AG genotype in Tolaki chicken, providing evidence that GH gene might be an important candidate gene for production traits.

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Keywords: Tolaki chicken; genotyping; GH gene; and association.

1. Introduction

In the tropics the high prevalence of disease and the limited effort to improve the genetic quality are a major factor limiting the productivity of local chickens [1, 6]. Indonesia local chickens have a huge potential to be developed. The genetic potential was reflected by the enormous genetic diversity especially with regard to the adaptive traits, the ability to survive harsh conditions and under minimum feeding regimes [10]. The high diversity of local chicken traits is genetic materials that can be utilized to create preeminent chicken through directional selection focus on the traits with high economic value such as meat and production, adaptability and immunity.

Tolaki chickens are one of Indonesia native chickens wich have specific appearance. Tolaki chickens are indigenous in Southeast Sulawesi and the habitat is around the forest. They have similar traits with red junglefowl (Gallus-gallus) such as feather colour, aggressiveness and some antiviral traits. a posture of Tolaki chicken have relatively smaller and slimmer body shape than Kampong chicken. The average weight of cocks are 1.60±0.29 kg and hens are 1.29±0.21 kg [8]. The previous research was reported that the TLR4 gene and Mx gene were found in Tolaki chicken [7, 14].

Some previous research have been characterized the genetic of chicken. These results showed that that GH gene could be a genetic locus or linked to a major gene significantly affecting the growth and carcass traits in chicken [2]. Some studied cGH existence of native chickens in Indonesian. The study was to identify the characteristics and detect mutations on the gene cGH site. The samples used were kampung, walik, arab and nacked neck chicken.DNA extraction and amplification process were followed by DNA sequencing of cGH gene. There were 19 amplified samples. Amplification of cGH gene resulted fragment with length of 528 bp [11].

Molecular technique by selecting genotypes candidate genes associated with productivity and correlated with disease resistance properties to be used as Marker Assisted Selection (MAS) is expected to selection accelerate the formation of a superior strain of native chicken. This research aim to identify and study the polymorphism of cGH gene in Tolaki chicken. The genotype of cGH product was verify through the association between GH gene polymorphisms in Tolaki chickens with production traits. It was done by genotyping of GH gene and analysis the association with some indicator of production and vitality traits.

2. Materials and Methods

The research was conducted during two month (April to November 2015), at Laboratory of Poultry Breeding Faculty of Animal Science Halu Oleo University Kendari, Laboratory of Animal Breeding and Molecular Genetics Faculty of Animal Science, Bogor Agricultural University, Bogor.

2.1 Animal Experiments and Rearing

A total of 58 Tolaki chickens were used in this study**..** The Tolaki chickens were randomly placed at cages. The

cage size was 60 x 60 x 40 cm^3. All of the cages placed in a pen (5 x 15 m^2). The feed was a commercial feed for growing hens containing 18%-19% of crude protein and 2850 kcal metabolizable energy. Feed and water were given *ad libitum*.

2.2 cGH Gene Genotyping

All of the Tolaki chickens were genotyped using cGH gene as a marker. The blood sample was taken from the brachial vein in the wing area. Extraction of DNA used phenol-chloroform method [9]. DNA has been extracted in the PCR amplification. Specific primers were used to amplify the cGH gene with a foward primer (5 'GTCCGTGCTCTTCTCTTATC..3' and reverse primer (5 'GCGCAGGCTTCCATCAGTAT.3') [2]. RFLP method was used to determine the ge notype cGH gene. PCR product of cGH gene fragments cut by *Eco*RV restriction enzymes. PCR products were separated by electrophoresis 2% agarose gel. The frequency of genotype GH gene was calculated based on genotyping results.

2.3 Fenotyping

A total parental of 30 Tolaki chickens (25 hens and 5 cocks) were reared during four week and total produced 58 Tolaki chicken offspring. Fenotyping was conducted to obtain the data and the recording of production traits was obtained through body weight gain, feed intake, FCR, and mortality information.

2.4 Data analysis

Association of genotype with the observed variables was analyzed with Anova for completely randomized design using General Linear Model (GLM). GH genotype was as treatment, and production or antiviral variables data were as response. The genotype difference of each gene compared using Tukey's test at 5% level. The GLM procedurs of Minitab with the model :

$$Yij = \mu + Pi + eij$$

Where: Yij = production or antiviral traits, μ = overall mean, Pi = fixed effect of the cGH genotype or sex, and eij = random error [5].

3. Results

3.1 The Polymorphism of cGH Gene Genotype in Tolaki Chickens

Genotyping result was performed the length of PCR product in GH gene intron 3 was 399 bp. The PCR-RFLP of Mx gene was presented in Figure 1.

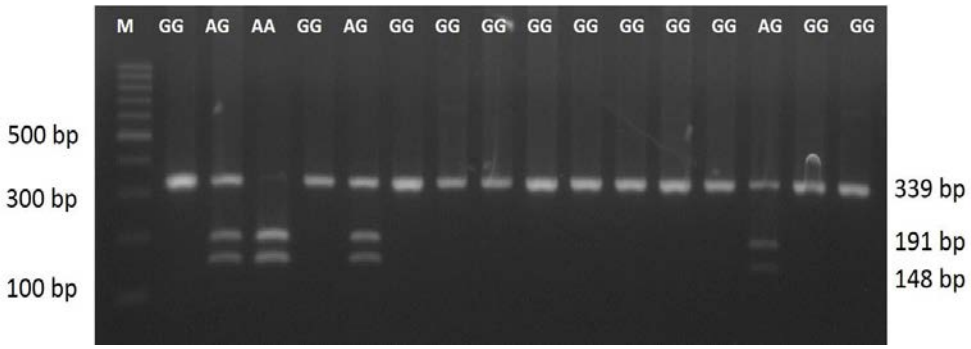

Figure 1: PCR-RFLP amplification product of cGH gene on intron 3 was cutted by *Eco*RV.

Figure 1 was the result of PCR of cGH gene fragment (399 bp) that was digested by EcoRV restriction enzyme in intron 3 (GAT|ATC). It was generated two allele (A and G) and three genotype (AA, AG and GG). The A allele was not digested by *Eco*RV so that produced one fragment (399 bp), whereas the G allele digested by *Eco*RV and produced two fragment (191 bp and 141 bp).

3.2 Association of sex with production trait and vitality of Tolaki chicken

The association of sex with production in Tolaki chickens was presented in Table 1.

Table 1: Association of sex with production trait and vitality of Tolaki chicken

Obesrvation of Traits	Genotype	
	Cocks (5)	Hens(25)
Production Traits		
Feed intake (g/head/d)	86,11[a] ± 2.60	82,64[b] ± 2.58
Weight gain daily(g)	8.16 ± 2.56	8,06 ± 2.46
Feed conversion	10,51 ± 1.54	10,25 ± 2.21
Vitality (%)	100	92

Note : Different superskrip letter in the same row indicate significant differences (P<0.05)

Association of GH gene with production traits and vitality in Tolaki chickens was presented in Table 1. The total number of samples were 30 parental of Tolaki chickens with cocks (5) and hens (25).

3.3 Association of cGH|EcoRV Gene with Production Traits

Association of cGH gene with production traits and vitality in Tolaki chickens was presented in Table 2

Table 2: Association of cGH/*Eco*RV gene genotype with production trait and vitality of Tolaki chicken

Parameter yang diamati	Genotipe		
	AA (1)*	AG(6)	GG(51)
Weight gain daily(g)	3,78	3,33[a] ± 1.91	4,15 [b] ± 1.58
Feed intake (g/head/d)	19.34	19,27 ± 0.61	18.57 ± 0.69
Feed conversion	5,11	5.77[a] ± 1.05	4.47[b] ± 1.58
Vitality (%)	100,0	83,3	100,0

Note: Different superskrip letter in the same row indicate significant differences (P<0.05)

- Production traits of AA Genotype were not included in the statistical tests

Association of cGH gene with production traits and vitality in Tolaki chickens was presented in Table 2. The total number of samples were 58 of Tolaki chickens with AA genotype (1), AG genotype (6) and GG genotype (51).

4. Limitations

The main problem in the development of native chicken is still limited effort serious and continuous to take advantage of native chicken as a raw material of genetic order to form a superior strain of chicken, whereas native chicken has the genetic potential of high economic value such as meat and egg production, the ability to adapt the tropical climate hotter, as well as resistance to viral diseases. Utilization of native chickens in Indonesia is still in early stages of cultivation, the chicken is only used as a final stock of meat and eggs.

The several of data and information of native chickens has not been well documented. Data and information on the economic characteristics of native chickens required for the purposes of selection of targeted and sustainable in order to maximize the productivity of native chickens.

5. Discussion

Figure 1 was the result of PCR of cGH gene fragment (399 bp) that was digested by *Eco*RV restriction enzyme in intron 3 (GAT|ATC). It was generated two allele (A and G) and three genotype (AA, AG and GG). The A allele was not digested by *Eco*RV so that produced one fragment (399 bp), whereas the G allele digested by *Eco*RV and produced two fragment (191 bp and 141 bp).

The genotyping result was detected G/A base mutation in the nucleotide of DNA GH gene. SNPs on GH gene are detected by PCR-RFLP. One G—A mutation is found in intron 3 for the first time, which can be clarified by digestion with EcoRV and confirmed by sequencing. The results of variance analysis show that it has a significant association with abdominal fat rate of chicken [2]. The result of PCR of cGH gene fragment (399 bp)

it was shortly fragment with cGH gene found in others native chicken of Indonesian (528 bp) [11]. so the cGH gene of Tolaki chicken was polymorphic and specific.

The association of sex with production in Tolaki chickens was presented in Table 1. The result showed that the weight gain and feed convertion in Tolaki chicken with cock were not significantly different with hens (P>0.05). However, it was significantly different (P<0.05) in the feed intake. The cocks have high feed intake than hens.It was correlation with weight gain daily The vitality of the both of parental Tolaki chicken was different. It mean that cocks (100%) showed better performance than hens (93%), these caused the hens need much more energy and protein that will used for egg production [13].

The results showed that the weight gain daily and feed conversion in Tolaki chicken with GG genotype were significantly different with AG genotype (P<0.05), but it was not significantly different in the feed intake. It mean that GG genotype showed better performance than AG, (Table 2).

These result indicated that there was correlation between productivity with immunity traits [3, 6] because the animal that could against disease attacks performed better production. The group of growth factor genes (GH, GHR, and IGF-1) influenced directly production trait [4, 12].

The results of vitality analysis showed that the vitality in Tolaki chicken with GG genotype (100%) was higher than AG (83%). The highest vitality of Tolaki chicken with GG than AG genotype was positive correlation with the productivity. In this case, Beside of GH gene influenced directly production, the performance of Tolaki chicken was influenced non-directly by Mx gene. The Mx gene could eliminated *Newcastle Disease viral* (NDV), consequently the NDV did not infected some the vitality organ of chicken.. Thus it could be caused the metabolism of chicken was optimally [7].

6. Conclusion

The association of cGH gene polymorphism with production, feed conversion, daily weight gain and vitality have been described in Tolaki chicken, GG genotype have better production and vitality than AA genotype in Tolaki chicken providing evidance that cGH gene might be an important candidate gene for production and vitality traits. However, this study has to be validated in other animal populations in order to evaluate its potential in selective breeding.

7. Recommendations

Results of genotyping cGH/*Eco*RV gene and production traits of Tolaki chicken was presented in this study. The data confirm that cGH gene genotype could be associated with production in tolaki chicken, This study recommend that cGH gene could be used as genetic marker selection in native chicken breeding program.

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