Identification

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Identification of GH Gene Polymorphisms and Their Association with Body Weight in Bayang Duck, Local Duck from West Sumatra, Indonesia

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Abstract. Polymorphisms in the growth hormone (GH) gene that is associated with the growth rate of duck have been the target of many breeding programmes. Molecular market selection has been an acceptable tool in the acceleration of the genetic response of desired to improve production performance in livestock. Growth hormone (GH) has been considered as a candidate gene for growth traits. In this study, polymorphisms of the GH gene were evaluated for associations with body weight in 210 Bayang duck. The objective of this study was to assess the association of polymorphisms in Growth Hormone (GH) genes with growth in Bayang ducks. The polymorphism of the ducks GH gene from promoter region into exon 3 was researched by polymerase chain reaction and DNA sequencing methods. Fifty duck were genotyped by sequencing twenty mutations were detected in the Bayang duck GH gene. Mutation G→A were detected at position -142, 1155, 1117, 1423, and 1786. Mutation C→T were detected at position -62, 506, and 308. Mutation A→G were detected at position 160, 264, 293, 1245, and 2542. Mutation T→G detected at position 250 and mutation TT→AA detected at position 350. Mutation G→T detected at position 775 and mutation T→C were detected at position 1353, 1424, and 2973. All polymorphism were polymorphics except polymorphism T→G at position 250 was monomorphics. Mutation G→A in position 1117 and 1786 were restriction with enzyme Eco721 and TscaI. In the case GH-Eco721 three genotypes were observed, GG, GA, and AA with frequency 0.041, 0.59 and 0.369 respectively. For GH-TscaI locus the frequency were 0.034, 0.782, and 0.184 respectively. The effect of GH-TscaI polymorphism were observed on body weight in 4 to 8 week of Bayang duck.

1. Introduction

West Sumatra, Indonesia has four species of local duck such as Pitalah, Kumbang Jonti, Kamang , and Bayang duck. All of this local duck are layer with small body size and lower meat production. Recently, the demand of duck meat in Indonesia are increasing together with the increasing of product diversification. To supply requirement and demand of duck meat, meat performance of some native duck breeds should be improved. Compare to poultry, duck price is more expensive, more stable and duck more resistant to the disease and its maintenance easier

In order to improve meat productivity of local duck genetics approach are needed to create superior breed by selection. These have led breeders to incorporate significant selection for increased growth rate in breeding programs by marker-assisted selection (MAS).To implement the MAS, markers are usually chosen in genes known to regulate the metabolic network controlling a particular quantitative trait [1]. Detection of candidate genes or markers responsible for the phenotypic variation in production traits remains a major challenge in genetic improvement programmes. Identification of

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direct markers is more useful than indirect markers for predicting the phenotypic superiority of the targeted traits of a population [2].

The application of molecular genetics in identification of polymorphism in growth candidate genes that show association with specific economically relevant traits provide useful information to enhance genetic improvement programme in livestock and validation of genetic markers of growth traits is the initial and crucial step to establish a Marker Assisted Selection system (MAS). The genes that are thought to have an influence on the growth of livestock include the Growth Hormone (GH), GHR, GHRL, and IGF1 genes that have been used as candidate genes in finding linkages between genotypes and phenotypes in livestock [3]. GH gene sequence in duck ducks have a length of 2162 base pairs (PB) consists of 5 exons and 4 similar introns in different mammal species.

Egg production and growth in chickens are influenced by many genes such as the GH gene [4] [5]. GH receptor: Insulin-like growth factor I. The GH duck gene is 5.25 kb in size, consisting of five exons and four introns, and is structurally similar to mammalian and chicken GH genes [6]. Furthermore, GH genes are very polymorphic in various livestock. Many polymorphisms have been identified in GH duck genes, for example in Pitalah ducks and Kumbang Janti duck [7]. , Peking and Mulard ducks [8], Tsaiya ducks, Muscovy ducks [9]. A previous study [7] found a very significant relationship between GH exon 1 gene and duck body weight at 5-8 weeks, whereas Wu et al [9] study found a relationships between GH polymorphisms and egg-laying performance. Thus also in the study Mazurowski et al [8] found a significant relationship between the diversity of GH exon 2 genes and the weight of Mulard ducks.

The genes that operate in the somatotropic axis mainly Growth Hormone (GH) is responsible for post-natal growth and development, tissue growth, lactation, reproduction as well as protein, lipid and carbohydrate metabolism. GH is an anabolic hormone synthesized in the anterior lobe of the pituitary gland and aid in body's immune response, wound healing and haematopoiesis. Current knowledge in production biology indicates that genetically superior animals differ from inferior animals mainly in their regulation of nutrient utilization and that GH exerts a key control in nutrient use. Those genes are thought to have influence on the growth of cattle Genes which are Growth Hormone (GH), GHR, GHRL, and has been used as IGF1 gene candidates in finding the link between genotype with Phenotype in cattle [3].

Egg production and growth in chickens is affected by many genes such as GH gene [4,5], GH receptor [6]; Insulin-like growth factor I. Gh gene in ducks have long 4350 bp of intron and exon 4 5 (GenBank: AB158760). GH on the ducks have a high diversity, Hiyama et al.[10] get 8 diversity in the gene promoter region of GH on ducks Mianmar. Kansanku et al., also reported the presence of diversity in the gene promoter region GH in Peking duck. Gene diversity is demonstrated by the presence of polymorphism on the site specific sites that may be linked to the gene expression in the nature of production. If the gene polymorphism is associated with the nature of the production, this certainly can be used as a tool of Marker Assisted Selection (MAS). Although the GH gene of duck has been cloned and sequenced in previous study. SNP polymorphisms of this gene and its marker-trait association analyses in Bayang ducks have not yet been reported. Therefore, the purpose of the current study was to identify and characterize SNPs in GH gene and then analyse the association between these polymorphisms and body weight in Bayang Duck. These SNPs would provide basic data for MAS in Local duck in west Sumatera, Indonesia. The objectives of this study were to identify the polymorphism of GH gene and to estimate the allele and genotype frequencies of GH-Eco721, GH-TsaIin Bayang ducks and possible associations of this two polymorphism with body weight of ducks in order to identify a potential marker to be used as a complementary parameter in the selection of ducks.

2. Material and Methods.

This research will be carried out on the technical implementation Unit and the Laboratory of Biotechnology Faculty of Animal Science, Andalas University, Indonesia. Field research aims to know the growth of duck. This research uses the 210 duck (110 male and 100 female). Body weights were originally weighed ducks and weights every week.

Blood samples (1 mL) were collected from the wing vein and transferred to tubes containing EDTA and then preserved at −30◦C. Genomic DNA was extracted using Promega Extraction Kit following the manufacture procedure. The quantity and quality of the extracted DNA was checked by Agarose gel electrophoreses. The fragment GH gene including exons 1 to exon 4 was amplified by designing three sets of primers using an online software tool "Primer3" input version 0.4.0 (http://primer3.ut.ee) Primer design based on duck GH gene sequence from the online database, the National Centre for Biotechnological Information (NCBI) (www.ncbi.nlm.nih.gov) (Gene accession number AB158760.2)

Table 1. Oligo sequence and position used for PCR growth hormone Gene.

Primer	Sequence	length (bp)	Tm
GHD1F	5'-CTGGAGCAGGCAGGAAAATT -3'	801	59°
GHD1R	5'-TCCAGGGACAGTGACTCAAC -3'	(exon1)	
GHD2F	5'- TGTGCCAGAGAGCAGAAGTT -3'	849	59°
GHD2R	5'-AGAGAGCTGTGAGGAGGAGA -3'	(exon2)	
GHD3F	5'- GGACAGCCTGAGGAAAGAGT -3'	834	59°
GHD3R	5'- GTGGAAGGTGGGGAGACTTC-3'	(exon 3 & 4)	

2.1. GH gene amplification

A total of 210 individuals of duck were successfully amplified by primers GHD1, GHD2, and GHD3 using 59°C annealing temperature. Amplification of the GH gene in this research resulted an amplicon with the length of 801, 849 and 834bp

The PCR reactions were performed with a total volume of 30 ul including 15 μL of the Master Mix from Thermo Scientific, 2 μL (20 ng) of genomic DNA, 1.5 μL (15 nM) of each primer (forward and reverse) and 10 μL of nuclease-free water. The cycling conditions were as follow:5 min at 94°C for initiation of denaturation, then 45 s at 94°C (denaturation), 45 s at 59°C (annealing), 60 s at 72°C for extension (35 cycles), followed by 5 min at 72°C for final extension. Amplication results of the PCR fragment was sent to 1st Base Singapore for sequencing.

2.2. Statistical analysis

The genotype and allele frequencies were calculated in each group of ducks. The data used to compare the effects of GH gene polymorphisms on duck body weight were tested with a model that included the effect of each genotype at the GH/Eco 721 locus. The genetic effects of the GH gene polymorphisms on body weight were analysed using a General Linear Model (GLM) procedure with model

$$Yijk = \mu + Gi + Sk + gijk \quad \ldots\ldots (1)$$

where, Yijkl is the observed value of the dependent variable, μ is the overall mean, Gi is the fixed effect due to genotype GH/Eco 721 (i = GH/CC, GH/CT or GH/TT), Sk is the fixed effect due to gender (k = males or females) and gijkl is the random residual error. The Hardy-Weinberg equilibrium was assessed with the Chi-square test. The statistical significance of differences among the means was calculated in accordance with the SAS/STAT Software, Release 6.12 (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussions

3.1. PCR product and sequencing

We used the sequencing method in order to assess and detect SNPs in Bayang ducks. We success fully amplified a fragment from promotor region to intron 4 of the cGH gene with 801, 849, and 834 bp in length in Bayang duck.

From the results of the product electrophoresis, all the samples were amplified well and it seemed that the fragments formed were specific because of the electrophoresis results, only one band formed with the desired length (801, 849, and 834bp). In order to see the sequence of the nucleotide fragments, the samples were then sequenced with a sequencer at 1st Base Singapore. Sequence results if compared to the sequence of dGH gene bank database has a high level of similarity with 97-100%. By comparing with the GH gene database sequence in the bank, showed 20 SNPs from promoter region to part of introns 4 (Table 2).Transition G→A were detected at position -142, 1155, 1117, 1423, and 1786. Transitions C→T were detected at position -62, 506, and 308. And transitions A→G were detected at position 160, 264, 293, 1245, and 2542. Transversion T→G detected at position 250 and mutation TT→AA detected at position 350. Transition C→T were detected at position 506, and 1308. Transversion G→T detected at position 775 and transition T→C were detected at position 1353, 1424, and 2973. All polymorphism were polymorphics except polymorphism T→G at position 250 was monomorphics. From this result it can conclude that GH gene oh Bayang duck is very polymprmics.

Polymorphism in position 1117, and 1786 than were genotype with PCR-RFLP with restricted enzyme Eco721, and TscaI. All sample (210) were amplified and restricted.

Table 2. Polymorphism GH gene in Bayang duck

No	Variationi	Position	Enzyme
1	G→A	-142	M*spI*
2	C→T	-62	A*luI*
3	A→G	160	Sekuensing
4	T→G	250	B*dsi*I
5	A→G	264	MboII
6	A→G	293	*Hpy1881*
7	C→T	308	Sekuessing
8	TT→AA	350	MboII
9	C→T	506	Sekuensing
10	G→T	775	Sekuensing
11	G→A	1117	*Eco*721
12	G→A	1155	Sekuensing
13	A→G	1245	*Hpy1881*
14	C→T	1308	*Bsaj*I
15	T→C	1353	*AocII*
16	G→A	1423	*Cac*81
17	T→C	1424	*Ajn*I
18	G→A	1786	TscaI
19	A→G	2524	Sekuensing
20	T→C	2973	Sekuensing

An 801 amplified fragment from promotor region to part of intron 1 was subsequently digested with Eco721 enzyme (Thermo Scientific). The restriction enzyme digestions were performed using 20 μL of PCR product mixed with 5 U of the appropriate restriction enzyme. The digestion of this 801bp PCR product differentiated three alleles GHGG with 801 bp,GHGA with 801, 564, 1nd 237 bp and GHAA with 564 and 237 bp (Figure 1), while the digestion of 834 bp PCR product corresponding to exon 3 and 4 of GH gene with TScaI enzyme also differentiated three allele (GHGG, GHGA and

GHAA) (Figure 2). There is little information in the literature regarding the allele and genotypic frequencies of RFLPs in GH gene due to Eco721 and TscaI restriction enzyme.

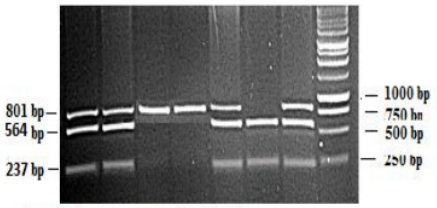

Fig. 1: *GH/Eco721* genotyp identification
Lane 1, 2, 5, 7 Genotyp GHGA 801, 564, and 237 bo, Lane 3 and 4 Genotyp GHGG 801 bp, Lane 6 Genotyp GHAA-564 and 237 bp, Lane 8 Molecular weight marker 250, 500, 750, and 1000 bp

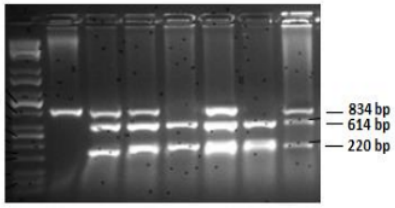

Fig. 2: *GH/TscaI* genotyp identification
Lane 1 Molecular weight marker-100, 150, 200, 300, 400, 500, 600, 800, and 100, Lane 2 Genotyp GHGG-834 bp, Lane 3,4,5, and 8 Genotyp GGGA-833, 614, 220 bp, Lane 5 and 7 Genotyp GGAA-614 and 220 bp

The allelic and genotypic frequencies of GHEco721 and GHTScaI polymorphic sites in Bayang duck populations are shown in Table 3. 1. The dominant allele of GHEco721 and GHTscaIof the duck GH gene was GHG. The allelic distributions of the GH/Eco721and GHTscaI polymorphic sites in the Bayang duck populations followed a similar pattern. As a result of digestion of an 801 bp target region of the duck GH gene from promotor to part intron 1 by the Eco 721 enzyme, the samples with an 801 bp fragment (uncut) were accepted as the GH/GG genotype, the samples with 801, 564 and 237 bp fragments were accepted as GH/GA and the samples with 564 and 237 bp fragments were accepted as the GH/AA genotype (Fig. 1) and the result of digestion of an 834bp target region of the duck GH gene from intron 1 to part intron 4 by the TscaI enzyme, the samples with an 834bp fragment (uncut) were accepted as the GH/GG genotype, the samples with 834, 614 and 220bp fragments were accepted as GH/GA and the samples with 614 and 220bp fragments were accepted as the GH/AA genotype (Fig. 2)

The genotype distributions of the GH gene in the studied duck populations are presented in Table 3. The most frequent genotype in the examined ducks were GH/GA. The genotype frequencies of the GH/Eco721 and GH/TscaIlocus in this duck population were not in Hardy-Weinberg equilibrium ($p>0.05$). The polymorphism GH/Eco721 were not association with body weight in Bayang duck but GH/TscaI, but The polymorphism GH/TscaI were association with body weight in Bayang duck.

Table 3. Allel and genotypic frequencies of GH gene in Bayang duck

Genes	N	Genotypic observation and genotypic frequency			Allee frequency	
GH/Eco721	210	GG 9 (0.043)	GA 124 (0.590)	AA 77 (0.367)	G 0.338	A 0.662
GH/TscaI	210	GG 9 (0.043)	GA 164 (0.781)	AA 37 (0.176)	G 0.433	A 0.567

Table 4. Association of GH/TscaI polymorphism with body weight at age 1-8 weeks in Bayang

Genotype	Body weight (g) at age (weeks)							
	1	2	3	4	5	6	7	8
GG	48.200	102.400	182.60	319.600	440.480	528.240	610.600	749.000
GA	45.500	105.697	213.424	363.333	495.409	585.742	680.985	807.363
AA	46.000	106.702	215.434	370.023	498.505	590.632	690.234	810.450
P value	NS	NS	NS	<0.05	<0.05	<0.05	<0.05	<0.05

In recent years, DNA polymorphisms have been widely studied in the GH gene of various animals. In this study, we detected SNPs in duck GH gene and analysed their association with body weight in Bayang duck. Twenty point mutations were identified from promotor to part intron 4. Previous research in GH gene have done by some researcher. Mazurowski et al. [8] found polymorphism in exon 2 than can detected by enzyme BsmI (GH/BsmFI). The influence of SNP in somatotropic axis–related genes on poultry reproduction is significant. Single nucleotide polymorphisms located in the coding region of GH were associated with fertility rate and the maximum duration of fertility in Tsaiya ducks and [2] found mutations in the GH gene were significantly associated with egg number in 52 week. GH polymorphisms and expression patterns in the muscovy duck and indicated a potential regulatory effect of GH on reproduction [9]. Three SNP were found in exon 4 of growth hormone gene in various China local duck that were related to some duck production characters [11].

In the present experiment the polymorphism of duck GH gene was examined from promotor region to part of intron 4. Previous studies had shown that the polymorphisms of the GHgene detected in ducks) and geese [12]. Meanwhile, polymorphisms in intronic regions of the avian GH gene were found in chickens at intron 1 [13]., at intron in ducks [9] (Wu et al.2012), at intron 3 in geese and chicken but also at intron 4 in chickens. There is little information in the literature concerning identification of GH-Eco721 polymorphisms of duck GH gene. Result of these result are contrary with our previous research, Ref. [7]) found GH-Eco721 polymorphism in Kumbang Janti duck and Pitalah duck that association with body weight in 5 – 8 week old. There is no information in the literature concerning of GH-TscaI polymorphisms of duck GHgene that can be compare with these study.

Conclusions

This study is the first research of the GH gene in Bayang ducks. This study discovered some new polymorphism of the GH. Polymorphism in position 1117 of these gene was not associated with body weight in Bayang ducks. But GH. Polymorphism in position 1786 was associated with body weight in Bayang ducks. Polymorphism in position 1786 can be used for genetically assisted selection in these breeds. Further study should be done to explore the association of other polymorphism with body weight of Bayang duck These findings are important as a basic information of Bayang duck Gh gene polymorphism.

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