Research Article

Polymorphism of Insulin_like Growth Factor -1 (IGF-1) Gene And Its Association with Growth Traits of Alope Chicken

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Abstract | This study aimed to identify polymorphisms of the (IGF-1) gene in Alope chickens and its relationship to the growth traits of Alope chickens. A total of 120 animals consisting of 52 cocks and 68 hens, were included in this study. Chickens were kept in individual cages to observe growth traits, including initial body weight, final body weight, daily body weight, body weight gain, feed consumption, and feed conversion ratio. Growth traits were analyzed using the general linear model method (GLM). To identify polymorphism using the PCR-Restriction Fragment Length Polymorphism (PCR-RFLP). Genotype frequency, allele frequency, and Hardy-Weinberg equilibrium were analyzed in this research. The research showed that three genotypes, AA, AB, and BB, were successfully visualized. The AB and BB genotype of the IGF-1 gene was significantly related (P<0.05) with final body weight, feed consumption, and feed conversion ratio in hens Alope chicken. So the IGF-1 gene has the potential to be used as a genetic marker for the initial selection process of Alope chickens.

Keywords | Alope chicken, Growth traits, IGF-1, PCR-RFLP, Polymorphisms

Received | May 01, 2024; **Accepted** | July 11, 2024; **Published** | October 05, 2024
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Citation | Tunnisa R, Dagong MIA, Purwanti S, Bugiwati SRA, Pakiding W (2024). Polymorphism of Insulin_like Growth Factor -1 (IGF-1) Gene And Its Association with Growth Traits of Alope Chicken. Adv. Anim. Vet. Sci. 12(11): 2205-2210.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.11.2205.2210
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Insulin-like growth factor 1 (IGF-1) is physiologically important for controlling livestock growth, development, metabolism, and lactation (Eom and Kim, 2024). IGF-1 is essential for the proper operation of many organs because it promotes synthesis, differentiation, and protein metabolism. It regulates differentiation by preserving differentiated function in several distinct tissues and a particular type of cell. Additionally, IGF-1 affects several tissues' anabolic and mitogenic effects of growth hormones (El-Tahawy and Abdel-Rahman, 2020; Laron, 2001; Ali *et al*., 2016; Yadav *et al*., 2023). The liver is the main source of IGF-1, although it is also produced in a specific manner in a tissue (Etherton, 2004).

Insulin-like growth factors (IGF) system is a complex system of peptide hormones (IGF-1 and IGF-II), cell surface receptors, and circulating binding proteins. IGF-1 and IGF-II bind to the insulin-like growth factor 1 receptor and in

Table 1: Primer sequence of IGF-1 gene.

Gene Target	DNA Sequence	Annealing Temperature	Restriction Enzyme	Amplicons
IGF-1	F: 5´-GAC TAT ACA GAA AGA ACC CAC-3´	55°C	PstI	621 bp
	R: 5´-TAT CAC TCA AGT GGC TCA AGT-3´			

Source: (Abbasi and Kazemi, 2011).

sulin receptor, activating their intrinsic tyrosine kinase domain activities. Several studies have shown that circulating IGF-I affects poultry's growth rate, body composition, and lipid metabolism (Zhou *et al*., 2005). Additionally, IGF-1 regulates the genetic variety of features like body size, average daily weight gain, live and carcass weight, efficiency in preserving food, fat deposition, and milk production (Amills *et al*., 2003).

IGF-1 is also significant for growth in domestic livestock animals. Growth is controlled by a complex system in which the somatotropic axis plays a significant role. GH and IGF-1 genes control the somatotropic axis and are responsible for postnatal growth.IGF-1 mainly mediates the function of GH, which acts on the growth of muscles and bones. Candidate genes have biological effects on physiology and development of traits as such genes instruct structural protein in biochemical and regulatory pathways by influencing on expression of traits (Eom and Kim, 2024). Based on several studies above, this study aims to identify the polymorphism of the IGF-1 gene in Alope chickens and its Association with growth traits that will allow it to become a genetic marker for selection based on superior traits.

MATERIALS AND METHODS

Animals

The study involved a total of 120 Alope chickens consisting of 52 cocks and 68 hens. Chickens are kept in individual cages from day-old chick to 70 days old.

Feeding Management

Feeding is done in the morning and evening ad libitum. The feed is a commercial feed consisting of corn, wheat flour, soybean meal, meat, bone meal, corn gluten, wheat bran, wheat bran, poultry product meal, DDGS, and palm oil.

Growth Traits Data Collection

The collection of growth trait data will later be used as data associated with genetic information. Data were collected in the following ways (Osei *et al*., 2013; Fahruddin *et al*., 2016): Initial body weight is measured at DOC hatching, measurement of final body weight is done by weighing the final weight when harvested, feed consumption is calculated based on the amount of feed consumed per day by looking at the recording every week, feed conversion is calculated based on the ratio between total feed consumption and end-of-week body weight (harvest weight).

DNA Isolation and Genotyping

A total of 2 mL of blood sample was taken via the axillary vein on the wing and collected in a vacuum container containing EDTA as an anticoagulant (Sambrook et al., 1989). It is then extracted according to the Genomic DNA minikit (Blood Culture Cell) protocol.

PCR-RFLP Amplification and Genotyping

A total of 2 µL of pure extracted DNA was put into a PCR tube to which master mix, H_2O, and IGF-1 gene primers consisting of forward and reverse were added and then put into the PCR machine. Table 1 shows the primary sequence. The amplification process consists of three stages. The process takes approximately 35 cycles using a PCR machine (SensoQuest, Germany). The amplification stage for the IGF-1 genes begins with initial denaturation at 94°C for 10s, and the annealing temperature for each can be seen in the table above and lasts for 30s. The final stage is an extension using a temperature of 72°C for 30s.

The Restriction Fragment Length Polymorphisms (RFLP) technique was used to determine the IGF-1 gene genotype. The IGF-1 gene uses the *PstI* restriction enzyme with a cleavage site. A total of 5 µL of PCR product was transferred into a 0.2 mL tube and added with 0.3 µL enzyme, 0.7 µL buffer and 1 µL H_2O. The product was homogenized using a vortex, centrifuged at 1.000 rpm for 1 min, and then incubated for 19h at 37°C.

Visualization of DNA Fragments

PCR-RFLP products were electrophoresed using 2% agarose gel. A 2 µL of PCR-RFLP product was mixed with loading dye, and fluorescein was homogenized and then put into the agar well that had been printed. Electrophoresis lasted for 45 min with a voltage of 105 volts. The genotype is determined by how many DNA bands appear after being visualized using a UV transilluminator. The DNA bands that appear are compared with markers to determine the length of the fragment, which is considered one type of allele.

Data Analysis

Allele frequencies, genotype frequencies, heterozygosity values, and Hardy-Weinberg equilibrium were estimated using Popgen 32 software (Yeh *et al*., 1999). Allele, geno

Table 2: Genotype frequency, allele frequency, heterozygosity, and Chi-square of IGF-1 gene.

Sex	N	Genotype Frequency			Allele Frequency		Heterozygosity		Chi-Square (χ^2)
		AA	AB	BB	A	B	H_e	H_o	
Cocks	52	0.10(5)	0.27(14)	0.63(33)	0.23	0.77	0.73	0.27	3.34
Hens	68	0.08(5)	0.30(24)	0.63(43)	0.22	0.78	0.70	0.30	1.59
Total	120	0.083(10)	0.28(34)	0.63(76)	0.22	0.77	0.72	0.28	4.44^{ns}

N: total of samples; **(..):** total samples of genotypes AA, AB, and BB; **Ho:** Observed Heterozygosity; **He:** Expected Heterozygosity; **ns:** Non-significance at P<0.05; (χ^2 **table:** 3.84).

type frequencies, and heterozygosity values were calculated using the procedure (Nei and Kumar, 2000). HWE was calculated using the following approach (Hartl and Clark, 1997). The association between genotype and growth traits was estimated using the General Linear Model (GLM) and Duncan's Multiple Ranges Test. Data was calculated using the Minitab 19. The mathematics model was (Hou *et al*., 2020):

$$Y\_ij=\mu+G\_i+\in\_ij$$

Description:
Yij= dependent variable for traits measured in the population,
μ = the mean of the population,
G_i = the genotype's fixed impact,
E_{ij} = the residual error.

RESULTS AND DISCUSSION

Polymorphism of IGF-1 Gene

IGF-1 gene polymorphism was identified in the Alope chicken population in this study. There are several genotypes, namely AA (257 and 364 bp), BB genotype (621 bp), and AB genotype (621, 364, 257 bp), according to the PCR-RFLP results (Figure 1). The purpose of looking at genetic polymorphism is to identify quantitative trait loci that have an impact on productivity and optimize breeding techniques (Edea *et al*., 2017).

The frequency of genotypes, alleles, heterozygosity values, and hardy-weinberg equilibrium of the IGF-1 gene (Table 2). Homozygous AA genotypes in cocks and hens Alope are (0.10 and 0.08) BB genotypes (0.63 and 0.63), and heterozygous AB in cocks and hens are (0.27 and 0.30). In Alope cock, the highest genotype frequency was BB (0.63), and the lowest genotype frequency was AA (0.10), as well as in Alope hens. This is evidenced by the frequency of the B allele in cocks and hens being higher than the A allele. The same results were obtained from the research (Ali *et al*., 2016), which found that the frequency found in Desi chickens was higher in allele B (55%) than in allele A (44%). It has been explained that a single nucleotide polymorphism (SNP) is polymorphic if the allele frequency value is ≤0.99 in large populations and ≤0.95 in small populations (Allendorf *et al*., 2013; Pandey *et al*., 2013).

In Table 2, the heterozygosity value is used as an indicator of genetic diversity (Nei and Kumar, 2000). The results of the analysis show that the observed heterozygosity value in Alope cocks is 0.27 and the expected heterozygosity is 0.73, while for Alope hens, the observed heterozygosity value is 0.30, and the expected heterozygosity is 0.70. When viewed based on the total population, the observed heterozygosity value of 0.28 is the same as the genotype frequency value, and the expected heterozygosity value is 0.72. If the Ho value in a population is lower than the He value, then the population indicates inbreeding (Nassiry *et al*., 2009).

The chi-square value of the total population (4.44) is greater than the X^2 table value of 3.84. This identifies that the Alope chicken population deviates from the assumption of Hardy-Weinberg equilibrium, where the Hardy-Weinberg law states that in a large population and conditions without natural selection, mutation, migration, and random mating, allele and genotype frequencies will remain constant from generation to generation (Graffelman, 2022). One of the causes of the possibility of deviating from the HWE equilibrium is mutation, migration, and inbreeding in the Alope population.

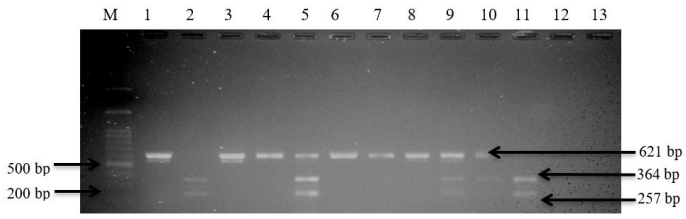

Figure 1: RFLP results of IGF-1 gen | PstI [M= Marker, Alope samples; genotype AA (samples 2, and 11 with 257, and 364 bp); genotype AB (samples 5, 9, 10 with 621, 364, and 257 bp); and genotype BB (samples 1, 3, 4, 6, 7, 8 with only 621 bp)].

Association of the IGF-1|PstI Gene on the Growth Characteristics of Alope Chickens

The parameters of growth traits in Alope chickens based on IGF-1|PstI genotypes are presented in Table 3. No statistically significant differences were found between the three genotypes regarding the overall parameters. The absence of genotype associations on growth traits is influenced by several factors, namely genetic complexity, environment

Table 3: Association of the IGF-1 gene with the growth traits of Alope chickens.

Parameter	Genotype			P-Value
	AA(10)	AB(34)	BB(76)	
Initial Body weight (g)	31.73 ± 4.77	32.135 ± 4.583	31.99 ± 4.006	0.960
Final Body Weight (g)	938.2 ± 147.7	996.1 ± 134.9	998.8± 126.2	0.38
Daily Weight Gain (g)	12.95 ± 2.081	13.77 ± 1.934	13.68 ± 2.264	0.556
Body Weight Gain (g)	906.5 ± 145.7	964.0 ± 135.4	958.1 ± 158.5	0.556
Daily Feed Intake (g)	44.89 ± 1.690	44.38 ± 1.703	44.86 ± 1.696	0.386
Feed Intake (g)	3142.9 ± 118.3	3106.9 ± 119.2	3140.8 ± 118.7	0.386
Feed Consumption Rate	3.435 ±0.613	3.169 ± 0.409	3.198 ± 0.462	0.267

(..): total samples of genotypes AA, AB, and BB.

Table 4: Association of the IGF-1 gene with the growth traits of Alope chickens based on sex.

Sex	Parameter	Genotype (n)			P-Value
		AA (5)	AB (14)	BB (33)	
Cocks	Initial Body weight (g)	33.36 ± 5.62	31.94 ± 5.09	31.95 ±4.06	0.082
	Final Body Weight (g)	1070.4 ± 57.3	1077.6±119.3	1053 ±114.1	0.782
	Daily Weight Gain (g)	14.81 ± 0.767	14.93 ± 1.708	14.59 ± 1.630	0.785
	Body Weight Gain (g)	1037 ±57.3	1045 ± 119.5	1021 ± 114.1	0.785
	Feed Intake (g)	3107 ± 95.1	3168 ± 12.2	3132 ± 104.2	0.487
	Daily Feed Intake (g)	44.40 ± 1.359	45.25 ±1.89	44.74 ±1.489	0.487
	Feed Consumption Rate	2.913 ± 0.243	2.97 ± 0.335	3.00 ± 0.357	0.823
		AA (5)	AB (20)	BB (43)	
Hens	Initial Body weight (g)	30.1 ±3.60	32.27 ± 4.32	31.9 ± 4.01	0.556
	Final Body Weight (g)	806 ± 46.1^{b}	939.1 ±116.5^{ab}	957.0 ±119.9^{a}	0.027
	Daily Weight Gain (g)	11.08 ± 0.678	12.95 ± 1.672	12.99 ± 2.488	0.179
	Body Weight Gain (g)	775.9 ±47.4	906.8 ±117.0	909.4 ±171.4	0.179
	Feed Intake (g)	3178 ±139.2^{ab}	3064 ± 89.6^{b}	3147 ± 129.5^{a}	0.027
	Daily Feed Intake (g)	45.40 ±1.989^{ab}	43.73 ± 1.279^{b}	44.96 ±1.850^{a}	0.027
	Feed Consumption Rate	3.95 ± 0.327^{a}	3.308 ± 0.405^{b}	3.344 ± 0.484^{b}	0.016

Different superscripts on the same row indicate significances differences (P<0.05); **(..):** total samples of genotypes AA, AB, and BB.

(Mulder, 2016), sample size and research design, genetic variation, and differences in measured traits. Of the above factors, the most likely ones that affect the absence of associations in the Alope population are the sample size and research design. The sample used in this population is 120 Alope chickens in the category of low sample size in measuring genetic diversity in a population.

Then the association of IGF-1 gene genotypes with growth traits based on cocks and hen's sex in Alope chickens is shown in Table 4. From the initial body weight trait, the superior genotype in hens is AA (33.36 g) while the hens, genotype AB (32.27 g). There is no statistically significant effect. In hens, the IGF-1 gene is significantly associated with final body weight, where the value of the BB genotype is different from that of the AA genotype but not that of the AB genotype. Statistically, the BB genotype is superior. This is by research (Wang *et al.*, 2004) that the IGF-1 gene is associated with 2-month-old body weight in Wangzai Yellow hens.

The results of the three genotypes between male and female chickens showed no significant effect (P>0.05) on the traits of daily body weight growth and body weight gain. The factor that influences the difference in growth between males and females is sex hormones. This is a common phenomenon found in almost all eukaryotic animals. (Hosnedlova *et al.*, 2020). Statistical analysis showed that female Alope chickens with the IGF-1 Gene were significantly associated (P<0.05) with final body weight, feed consumption, and feed conversion traits. The final body weight value of genotype BB (957g) was higher than genotypes AB (939 g) and AA (806g). As for feed consumption, the genotype values of genotypes AB and BB were different but not different

from the AA genotype, and the good value of the feed conversion ratio is genotype AB. Statistical analysis showed that female Alope chickens with AB and BB genotypes were significantly superior in these traits. This indicates that the AB and BB genotypes appear most frequently in the Alope chicken population. Therefore, it is likely that the AB and BB genotypes are used as determinants of superior traits in the Alope Chicken population that can be used for selection and breeding purposes.

The limitations of this study are the small number of populations used can limit the potential for significant associations with growth traits, and the potential for bias in the interaction of genes and the environment; further research should consider the number of samples to be observed and optimize the potential bias that can arise so that the results obtained can contribute more to science, society about the potential of the IGF-1 gene as a genetic marker, especially in local chickens.

CONCLUSIONS AND RECOMMENDATIONS

Three IGF-1 genotypes were found in the local chicken population: AA, AB, and BB. The B allele is the common allele found in Alope chickens with a frequency of 0.77. The B allele is positively associated with the growth traits of Alope chickens, especially in final body weight, feed consumption, and feed conversion. Polymorphism of the IGF-1 gene in Alope chickens can be utilized as a molecular marker in selection.

ACKNOWLEDGMENTS

We thank the project team of Riset Inovasi Indonesia Maju (RIIM) batch 1 in 2023 for the research grant funding from BRIN.

NOVELTY STATEMENT

The authors declare the article original and sourced from unpublished research data. All experimental procedures on animals were performed according to the recommendations and approval of the Research Ethics Committee of Hasanuddin University (UH23040216).

Conflict of Interest

The authors have declared no conflict of interest.

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