Polymorphism analysis of the *Insulin-like Growth Factor-1* (*IGF-1*) gene encoding growth in F_5 Golden Kamper chicken (*Gallus gallus domesticus*)

Elysia Mutiara Azizah[1], *Nata* Dwi Annisa Nizma[1], *Patricia* Graciella Audrey Prajaningtyas[1], *Angga* Bintang Narzassi, and *Budi* Setiadi Daryono[1*]

[1]Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Sel., Sendowo, Sinduadi, Kec. Mlati, Kabupaten Sleman, Daerah Istimewa Yogyakarta 55281, Indonesia

Abstract. Golden Kamper Chicken is a chicken cross between pelung and layer chickens with good meat and egg productivity. Insulin-like growth factor-1 (IGF-1) gene is one factor affecting the speed of chicken growth. This research aims to study the character of F5 Golden Kamper chickens and analyze the IGF-1 gene to determine its effect on chicken growth. The research will be done by hybridizing the F4 Golden Kamper hen and rooster, raising brood stock and DOC, egg collection, growth data collection, blood collection, DNA isolation, DNA amplification, electrophoresis, and sequencing. The observed parameters are DOC weight for the first seven weeks and polymorphism of the IGF-1 gene. Data was analyzed using Microsoft Excel, Genestudio, MEGA11, and IBM SPSS 25 software. Sequencing data were processed using the Genestudio application, alignment with MEGA11, and genotype-haplotype associations using Pearson correlation and Linear Regression F test. The results showed that the molecular marker polymorphism of the IGF-1 gene was found at 12 mutation points. IGF-1 gene polymorphism did not correlate with the weight of F5 Golden Kamper chickens.

1 Introduction

The main consumption commodity of the Indonesian people that comes from the agriculture, forestry, livestock, and fisheries sectors is native or local chickens. Based on data from Badan Pusat Statistic, in 2022, native or local chicken production reached 314101.3 thousand chickens. Livestock production following the increase in public consumption of local chicken meats and eggs. Consumption of local chicken meat increased from 0.14 kg to 0.15 kg per capita every week. In addition, the average consumption of local chicken eggs in 2022 increased from 2 eggs to 3 eggs per capita each week [1]. The increase in the consumption of local chicken meat and eggs is due to Indonesia's population growth of as many as 3.1 million people. Indonesia's population growth in 2022 increased from 272.7 million to 275.8

* Corresponding author: bs_daryono@mail.ugm.ac.id

million people. Consumption of local chicken meat and eggs continues to increase due to the increasing Indonesian citizen population this year. This matter urges poultry farmers and researchers to maintain and create superior chicken strains. With these problems, the Gama Ayam Research Team from the Faculty of Biology, Universitas Gadjah Mada, maintains the Golden Kamper chicken strain. Gama Ayam Research Team is a research team that has succeeded in assembling F_1 Kamper Chickens from 2013 to 2014 [2]. F_1 Kamper Chickens is a crossbreeding chicken between Pelung rooster and laying hens (layers). Eggs and meat productivity of F_1 Kamper chickens is good. However, the color of this chicken feather is still quite diverse, with a high level of heterozygosity, so the Gama Ayam Research Team carried out an inbreeding cross on this F_1 Kamper chicken. In 2016, the Gama Ayam Research Team conducted crosses between rooster and hen of F_1 Kamper chickens (inbreeding) to produce a new chicken strain with a golden phenotype, which is called F2 Kamper, also known as the Golden Kamper chicken [3]. The weight of the chicken is one of the characteristics that show the productivity of the chicken, so weight measurements are carried out in chickens.

Currently, Golden Kamper Chicken has reached its fifth generation, namely F_5 GK (Golden Kamper 5th Filial). F_5 GK is very necessary to be studied to compare their growth and development with the previous generation of Golden Kamper chicken. The somatotropic axis regulates the growth and development of chickens. The somatotropic axis is the hypothalamic-pituitary growth axis, which consists of important compounds such as hormones. Therefore, it can be said that hormones are an important factor in the growth and development of chickens. These hormones include the *GHRH* hormone (Growth Hormone Releasing Hormone), *GH* hormone (Growth Hormone), *SS* hormone (*Somatostatin Hormone*), IGF-1 hormone (Insulin-like Growth Factor-1), IGF-2 hormone (Insulin-like Growth Factor-2), and others [4]. Chicken growth can be involved with the biological function of genes. Various genes control growth traits in chickens, one of which is the Insulin-like growth factor-1 (IGF-1) gene. The IGF-1 gene or somatomedin B factor 1 (SnaBI) gene is a gene produced by growth hormones in various body tissues, especially in the liver. The IGF-1 gene plays a role in the growth and development of bones and muscles [5]. This IGF-1 genomic structure consists of various parts or regions, i.e., exon 1, exon 2, exon 3, exon 4, and 5'UTR. One part of this gene, namely the 5'UTR region, affects the initiation of translation and ribosome [6]. Based on previous research, an association of IGF-1 promoter gene polymorphism with Average Daily Gain (ADG) was found in 2 genetically diverse Black Penedesenca chicken strains, and the IGF-1 gene can be a candidate gene that affects growth in broiler chicken [7]. Therefore, in this study conducted inbreeding between rooster and hen of F_4 Golden Kamper chicken to get F_5 Golden Kamper chicken and analysis of the *IGF-1* gene to determine its effect on the growth of F_5 Golden Kamper chicken.

2 Materials and methods

2.1 Materials

The object of this research is the hen and rooster of F_4 Golden Kamper, pelung and layer chickens, and 12 DOC (Day Old Chick) of F_5 Golden Kamper. Material needed in this research are BR-I type feed for DOC chickens (0-7 weeks old), AD-II type feed for adult chickens (>7 weeks old), water, egg stimulant Medion, vitamins, EDTA tube Vaculab® brand, alcohol 70%, ice gel, syringe, latex gloves, nitrile gloves, tissue shavings, medical masks, blood taken from DOC and parental, microtube, PCR tube, DTT, proteinase K, TE Buffer (pH 8,0), TBE Buffer (Tris-Borad EDTA 1X), 5% Chelex solution, distilled water, white tip pipette, yellow tip pipette, blue tip pipette, aluminum foil, plastic wrap, Bioline

Mastermix PCR (2X MyTaq HotStar Red Mix), *IGF-1* primer forward and reverse according to design (622 bp), ddH_2O (double-distilled water), Agarose gel, Florosafe DNA Stain, and 100 bp DNA ladder. The tools used in this research include adult chicken cages, DOC cages, places for drinking water and feed, egg trays, digital scales (semi-analytical scales) brand KrisChef EK9350H, camera (phone), stationery, incubator, incandescent lamp 50-watt bulb, calculator, ice box, freezer, refrigerator, microtube trays and PCR tubes, micropipettes, Erlenmeyer tubes, beaker glass, measuring cylinders, funnels, incubator tube, micro centrifuge, vortex mixer, spindown, Thermal PCR machine cycler (Biorad), microwave, heat blox, electrophorator, UV Transilluminator, and GelDoc, and also sequencing machine (Applied Biosystem 3500 Genetic Analyzer 2550).

2.2 Methods

2.2.1 Maintenance of chickens and collection of eggs

This research was conducted from July 2022 to February 2023 in PIAT UGM, Sawitsari Research Station Faculty of Biology in Depok, Sleman, Special Region of Yogyakarta, and also in the Hatching Machine Company of HTN Tirto Yogyakarta. Then, from March 2023 to June 2023, research continued in the Genetics and Breeding Laboratory, Faculty of Biology, Gadjah Mada University. One Hen ♀ F_4 Golden Kamper and one rooster ♂ F4 Golden Kamper are mated or hybridized by being kept in the same cage belonging to the Gama Ayam Research Team. The feed given to chickens is AD-II feed. Food and drink are provided every day by ad libitum. Eggs are taken every day from the cage and then in the eggshell given the date with pencil when the egg was taken. Every Thursday, the eggs that have been collected later put into an egg incubator with a capacity of 200 eggs swivel rack system. Chicken eggs are hatched for 21 days in the hatcheries machine located in the HTN Tirto Yogyakarta.

2.2.2 Maintenance of chick or Day Old Chick (DOC)

Chickens have been maintained intensively in the cage, which was given a 15-watt incandescent lamp for 24 hours so that the cage remained warm. Feed of BR-I and water are given every day by ad libitum. DOC were weighed every week for the first seven weeks.

2.2.3 Growth data collection

DOC weight was measured every week for seven weeks by digital scales (semi-analytical scales) brand KrisChef EK9350H.

2.2.4 Blood collection

Blood collection was coducted on parental chicken and 12 DOC that are seven weeks old (14 samples in total). Blood was collected through the brachial vein as much as 1 mL using syringes. The blood sample is transferred into the vacutainer with Ethylenediaminetetraacetic (EDTA) anticoagulants or brand-name EDTA tubes Vaculab®. The EDTA tube is stored in an ice box before being transferred inside the refrigerator in the Genetics and Breeding Laboratory, Faculty of Biology, UGM, with a temperature of -20°C.

2.2.5 DNA isolation

DNA isolation was carried out using Chelex method. A total of 10 µL blood sample from the EDTA tube was put into a 1.5 mL microtube, and then 1 mL TE Buffer (10 µM, pH 8) was added. Centrifugation at 13.000 rpm was carried out for 3 minutes on 14 microtube samples. The supernatant portion of the centrifugation results was discarded. While part of the pellet was taken and transferred to a new microtube. The pellet was added with 200 µL of 5% Chelex solution, 18 µL DTT 0.05 M, and 2 µL proteinase K 20 mg/mL. Vortex the mixture for 10 seconds and then incubated for 1 hour at 60°C. The solution of the incubation result has cooled. Then, that solution was centrifuged at 13.000 rpm for 3 minutes. A total of 150 µL supernatant was transferred into a new microtube and stored in the freezer at -20°C.

2.2.6 DNA amplification (Polymerase Chain Reaction / PCR)

DNA amplification materials such as 1.25 µL forward primer, 1.25 µL reverse primer, 8 µL ddH_2O, and 12.5 µL PCR Mastermix with a total reaction of 23 µL were put into the microtube then homogenized for use as a cocktail. Isolation results : 2 µL of DNA for each sample was taken and put into the PCR tube. Then, 23 µL of the homogenized cocktail was added. The sample (25 µL) was put into the PCR machine to be amplified for 2 minutes at a pre-denaturation temperature of 95°C. The cycle of denaturation stages is carried out at a temperature of 95°C for 30 seconds, the annealing stage at 58,5°C for 30 seconds, the elongation stage (extension) at 72°C for 30 seconds, and the end elongation stage (post-extension) at 72°C for 5 minutes. The cycle of denaturation, annealing, and extension was repeated 35 times. The primer design that was used with 622 base pairs length in this research are *forward* primer (5-GACTATACAGAAAGAACCAC-3) and *reverse* primer (5-TATCACTCAAGTGGCTCAAGT-3). A total of 14 PCR sample products were prepared for the electrophoresis method.

2.2.7 Electrophoresis

Electrophoresis material such as 1.5% agarose gel in 0.5X TBE buffer was prepared. As many as 0.8 grams of 2% agarose gel was taken and put into an Erlenmeyer. A total of 40 mL of 0.5X TBE was added to the Erlenmeyer. The mixture was then put into the microwave (600 watts) for 40 seconds until the agarose powder dissolved and boiled (around 75°C), then homogenized by shaking. After the mixture in the Erlenmeyer has cooled, 2 µL of florosafe is added and then poured on the comb until it thickens to form wells. TBE buffer 0.5X was added to drown the gel. A total of 3 µL of 100 bp DNA marker was poured into the first well, followed by 5 µL of PCR product. Electrophoresis was carried out at 100 V for 30 minutes. DNA fragments were visualized using UV transilluminators, GelDoc, and OptiLab 3.0 software. At this stage, 3 samples, namely 2 parental and DOC number 12, were not used for further processing, so the sequencing process was only for DOC samples number 1 to 11 (11 samples).

2.2.8 Sequencing

The DNA amplification product was prepared and placed in an ice box. The IGF-1 nucleotide sequencing process from the PCR results was carried out by laboratory assistant using the Sanger Sequencing method in the Integrated Research and Testing Laboratory, UGM (LPPT UGM).

3 Result and discussion

3.1 Weight growth of F_5 Golden Kamper chickens

This study focuses on the ability of F_5 Golden Kamper chickens as broiler chickens, so their weight growth is closely observed and data is collected. The study began by crossing a rooster F_4 Golden Kamper chicken with a hen of F_4 Golden Kamper chicken.

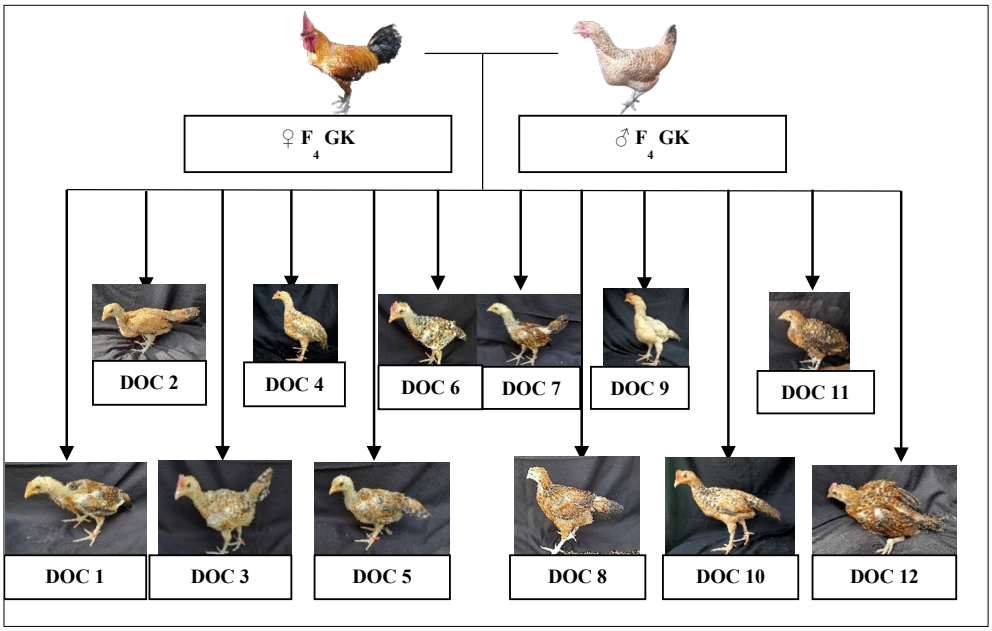

Fig 1. Pedigree of chickens ♀ F_4 Golden Kamper and ♂ F_4 Golden Kamper

The inbreeding of the F_4 Golden Kamper chickens produced 12 DOCs of F5 Golden Kamper chickens (Fig.1). A total of 11 of these DOCs had normal physical characteristics. However, DOC number 12 had a defect in its left leg since hatching. Despite this, DOC number 12 managed to live until 7 weeks old (49 days). Based on morphological observations in DOC of F_5 Golden Kamper, it was identified that there were male and female. The morphological differences between male and female chickens that were observed in this study were feather shape. Male chicken feathers are usually longer and pointed, while female chicken feathers are round and shorter [8]. Chickens with male gender are DOC numbers 2, 3, 4, 6, 8, 9, 10, 11, and 12, while chickens with female gender are DOC numbers 1, 5, and 7. Based on the results of this crossbreeding, it can be concluded that the number of DOC with male gender is higher than those with female gender.

The weight of the F_5 Golden Kamper DOCs was measured every Sunday from hatching until they were 49 days old. Based on these measurements, it was found that the weight of the F_5 GK DOCs increased every week. Each DOC shows a high increase in body weight every week, except for DOC number 1. The weight of DOC number 1 only gained 2 grams in the first week, and it was even worse in the second week, little to no weight was gained. It was possibly due to the lack of the DOCs immune system. The immune system of young chickens or DOCs has not fully developed at 6 to 13 days old, so adequate nutrition must be given until the DOCs are 30 days old [9]. DOC number 1 had the lowest weight among all

DOCs (260 grams). Meanwhile, the DOC with the highest weight in the final measurement week was DOC number 9 (585 grams).

Table 1. Weight of F_5 Golden Kamper chicken every week until 7th week

Sample code	Chicken code	Chicken Weight (gram)							
		Before a week	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week
1	DOC 1	38	40	40	52	74	107	232	260
2	DOC 2	42	63	98	154	200	258	310	386
3	DOC 3	49	65	80	131	166	225	254	428
4	DOC 4	47	60	122	186	266	355	359	409
5	DOC 5	38	43	53	62	85	134	250	306
6	DOC 6	45	72	77	99	141	203	272	364
7	DOC 7	37	47	58	72	99	150	270	310
8	DOC 8	49	56	83	123	165	219	305	431
9	DOC 9	51	74	128	217	293	418	509	585
10	DOC 10	44	68	125	201	297	395	406	431
11	DOC 11	42	68	129	191	289	326	371	450
12	DOC 12	42	86	135	176	238	310	322	447
	Average	43,67	61,83	94,00	138,67	192,75	258,33	321,67	400,58

3.2 Analysis of *IGF-1* Gene polymorphism on weight growth of F_5 Golden Kamper chicken

The F_5 Golden Kamper chickens, blood samples were collected for molecular testing. The blood collection location on the chicken's body is on the elbow of inner wing. The brachial vein was chosen as the location for blood collection because the size of the vein is large enough to facilitate the blood collection process. However, blood collection in this location must be done carefully because the location of the brachial vein is close to the median nerve and brachial artery. If not careful during the process, it can cause bleeding in the chicken. The blood samples are ready to be isolated. DNA isolation was done by the Chelex method.

Blood samples from 12 DOC and their parents ware prepared for DNA isolation. Prior to initiating the DNA amplification process, a preliminary quantitative assessment of the isolated DNA is conducted. The quantitative test of the isolated DNA was done using the NanoDrop Spectrophotometer machine (Thermo Scientific, USA). The sample with the highest concentration and purity of DNA isolate is DOC 6, while the lowest is DOC 12. The concentration of DNA isolate in DOC 6 is 720.321 ng/µL, while its purity level is 1.748. The concentration of isolated DNA in DOC 6 is 113.389 ng/µL, while its purity level is 1.261. The concentration of DNA isolate in all samples has met the minimum limit (100 ng/µL). However, none of the 14 DNA isolate samples meet the good purity range (1.8-2). The low purity can be caused by the sample's lack of clarity and solubility during isolation. The possibility of contamination from tools such as pipettes can also reduce the purity of DNA isolates [10].

After the DNA isolation sample has been prepared, the next method is DNA amplification. The primer design in chickens with the target gene region 5'UTR uses the

forward primer (5-GACTATACAGAAAGAACCAC-3) and reverse primer (5-TATCACTCAAGTGGCTCAAGT-3) along 622 bp (base pairs) [7]. The PCR Mastermix used in this study is Bioline Mastermix PCR (2X MyTaq HotStar Red Mix). MyTaq HotStar Red Mix is a red-colored PCR mastermix consisting of MyTaq DNA polymerase and buffer system. MyTaq Red Mix is used in this study because it efficiently provides good and quick results. Moreover, MyTaq DNA exhibits robust amplification capabilities, making it effective in handling challenging PCR templates, including those with excessively long or short primers. Distilled water (ddH_2O) here serves to prevent nucleic acid degradation and eliminate possible side reactions that interfere with results.

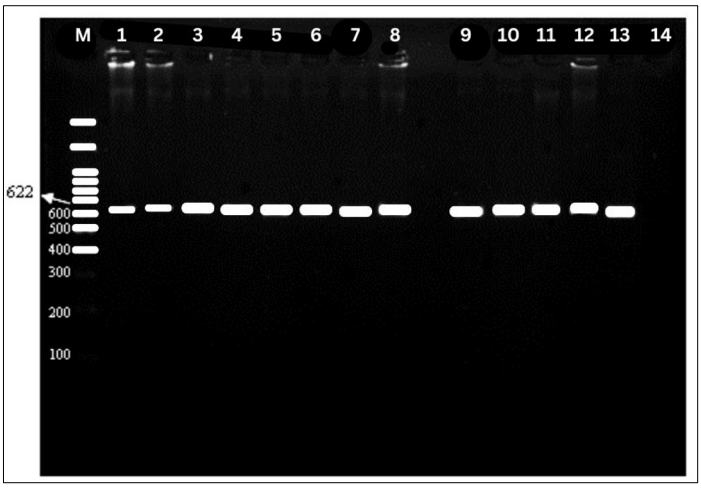

Fig 2. Result of IGF-1 gene visualization on 14 samples

Electrophoresis is carried out at a voltage of 100 volts for 30 minutes. Electrophoresis works by separating DNA samples based on their molecular weight, which indicates the number of base pairs produced during the amplification process. In electrophoresis, agarose gel and Florosafe DNA stain are used. The visualization results of the IGF-1 gene in rooster and hen parental samples and DOC F_5 Golden Kamper can be seen in figure 2. Two PCR products of the parental F_4 Golden Kamper and 11 DOC F_5 Golden Kamper can be visualized according to the 622 bp target. However, one DOC PCR sample, namely DOC number 12, could not be visualized. The absence of PCR results for DOC number 12 may be due to the low concentration and purity of the DNA isolate produced in the previous process. The PCR product of DOC F_5 Golden Kamper samples was submitted to LPPT (Laboratory of Research and Integrated Testing, UGM) for the Sanger sequencing stage.

The sequencing data of the IGF-1 gene of all samples were prepared for alignment. Contig editing was performed on all samples to clean up the electrogram of each sample. This editing process was done using Genestudio Pro software. Alignment was then performed on all sample sequences along with the wild-type chicken IGF-1 gene reference (EF198877.1) using the MEGA11 application. There were 12 polymorphism points observed from the alignment results of the Insulin-like Growth Factor-1 (IGF-1) gene in 11 DOC F_5 Golden Kamper chickens. These points were tabulated as data to determine the DNA sequence changes in each chicken sample and the resulting haplotypes. Based on the table 1, there are 12 polymorphism points in the IGF-1 gene alignment results on the 5'UTR region associated with the growth of F_5 Golden Kamper chickens. The types of polymorphism that occur are insertion and substitution mutations. The polymorphism points found include 42G insertion, 117C insertion, T205C substitution, T270C substitution, 276A insertion, 277G insertion, 278T insertion, 292A insertion, 293C insertion, T300G substitution, 237T insertion, and

C351T substitution. There are 3 points transition substitution, 1 point transversion substitution, and 8 points insertion. All individuals of DOC F_5 Golden Kamper (11 individuals) have polymorphism, which causes high levels of genetic variation in individuals. The other reason is maybe because the weight of one DOC to another DOC is not significantly different. In addition, polymorphism at the 12 points or positions occurs in the non-coding region (5'UTR region), so the polymorphism would not affect the chicken growth. Concurrently, alterations in nucleotide bases at the 12 specific positions within the 5'UTR region of the Insulin-like Growth Factor-1 gene impact translation initiation and ribosome function. Consequently, the polymorphism that occurs can cause changes in the function of DNA synthesis regulation [6].

Table 2. Point of IGF-1 gene polymorphism in DOC F_5 GK sequencing results

No	Polimorfism of *IGF-1* Gene	Sample of DNA Sequencing											
		***IGF-1* Gene Reference (EF198877.1)**	**DOC1**	**DOC2**	**DOC3**	**DOC4**	**DOC5**	**DOC6**	**DOC7**	**DOC8**	**DOC9**	**DOC10**	**DOC11**
1	Insertion 42G	-	-	-	-	-	-	-	G	-	-	-	-
2	Insertion 117C	-	-	-	C	-	-	C	C	C	-	C	-
3	Substitution T205C	T	C	C	T	T	C	C	T	C	T	C	C
4	Substitution T270C	T	C	C	T	C	C	C	T	C	C	C	C
5	Insertion 276A	-	-	-	-	A	-	A	A	-	-	A	-
6	Insertion 277G	-	-	-	-	G	-	G	G	-	-	G	-
7	Insertion 278T	-	-	-	-	T	-	T	T	-	-	T	-
8	Insertion 292A	-	-	-	-	-	-	A	-	-	-	-	-
9	Insertion 293C	-	-	-	-	-	-	C	-	-	-	-	-
10	Substitution T300G	T	T	G	T	T	T	T	T	G	T	T	T
11	Insertion 237T	-	-	-	-	-	-	T	-	-	-	-	-
12	Substitution C351T	C	T	T	C	C	T	T	C	T	C	T	C
	Haplotipe	Reference	1	2	3	4	1	5	6	7	8	9	10

3.3 Analysis of correlation coefficient and linear regression of *IGF-1* Gene polymorphism on weight growth of F_5 Golden Kamper chicken

After identifying the polymorphism point of 11 DOC F_5 Golden Kamper, correlation coefficient analysis and F Test (Simultaneous) Linear Regression were conducted using IBM SPSS 25 software to determine whether the polymorphism affects the growth of F_5 Golden Kamper chicken weight. The correlation coefficient test conducted in this data analysis is the Pearson correlation test. The Pearson correlation test is a simple correlation test or

measurement that uses two variables, independent or unbound variables, and dependent variables, to determine the positive or negative relationship between them.

Table 3. Result of Pearson correlation test and linear regression F test on IGF-1 gene polymorphism on the growth of DOC F_5 GK

Polimorfism	Genotype	Genotype Frequency	Chicken Weight in 49 days age (gram)	Pearson Correlation	Linear Regression F Test	Significance	Conclussion
Insertion 42G	G-	0,91	421,11	-0,326	1,067	0,329 (p>0.05)	No significant effect
	GG	0,09	310,00				
Insertion 117C	C-	0,55	427,20	-0,039	0,014	0,91 (p>0.05)	No significant effect
	CC	0,45	392,80				
Substitution T205C	TT	0,36	433,00	-0,033	1,101	0,321 (p>0.05)	No significant effect
	TC	0,64	394,67				
Substitution T270C	TT	0,18	369,00	0,154	0,218	0,652 (p>0.05)	No significant effect
	TC	0,82	420,25				
Insertion 276A	A-	0,64	431,00	-0,161	0,239	0,636 (p>0.05)	No significant effect
	AA	0,36	378,50				
Insertion 277G	G-	0,64	431,00	-0,161	0,239	0,636 (p>0.05)	No significant effect
	GG	0,36	378,50				
Insertion 278T	T-	0,64	431,00	-0,161	0,239	0,636 (p>0.05)	No significant effect
	TT	0,36	378,50				
Insertion 292A	A-	0,91	415,11	-0,122	0,136	0,721 (p>0.05)	No significant effect
	AA	0,09	364,00				
Insertion 293C	C-	0,91	415,11	-0,122	0,136	0,721 (p>0.05)	No significant effect
	CC	0,09	364,00				
Substitution T300G	TT	0,82	410,38	0,068	0,042	0,842 (p>0.05)	No significant effect
	TG	0,18	408,50				
Insertion 237T	T-	0,91	415,11	-0,122	0,136	0,721 (p>0.05)	No significant effect
	TT	0,09	364,00				
Substitution C351T	CC	0,45	436,40	0,436	2,109	0,180 (p>0.05)	No significant effect
	CT	0,55	383,60				

The Pearson correlation test is used to determine how strong and close the relationship between one variable and another variable. The degree of correlation coefficient is used to interpret and show the relationship or association between variables. The degree of relationship between two variables is commonly symbolized by 'r'. The correlation coefficient values are between $-1<0<1$. The values of -1 and 1 indicate a perfect negative relationship (inverse correlation) and a perfect positive relationship (direct correlation). If 'r' is 0, then the two variables do not have any relationship [11, 12].

Table 4. Result of Pearson correlation test and linear regression F test on IGF-1 gene polymorphism on the growth of DOC F_5 GK

No	Interval r (+/-)	Correlation Interpretation
1	0.00	No correlation
2	0.01-0.199	Very weak correlation
3	0.20-0.399	Weak correlation
4	0.40-0.599	Moderate correlation
5	0.60-0.799	Strong correlation
6	0.80-0.999	Very strong correlation
7	-1.00/1.00	Perfect correlation

The first polymorphism point, a correlation value (r) of -0.326 is obtained. According to table 3, the interpretation of the r value has a weak correlation. At the second to eleventh point of polymorphism, correlation values (r) were obtained of -0.039, -0.033, 0.154, -0.161, -0.122, and 0.068. The interpretation of these r values is to have a very weak correlation. The last polymorphism point, namely the twelfth point, has a correlation value (r) of 0.436. This value indicates that these points have a moderate correlation with the growth of chicken weight.

The Linear Regression F test was carried out with the aim of looking for the influence of the independent variable (free variable) on the dependent variable (dependent variable). The maximum limit of test error is 0.05 or 5%. The degrees of freedom (df) in this study are derived by subtracting one chicken from the total number of observations, which, in this case, corresponds to the number of chicken samples under investigation. Therefore, the degrees of freedom utilized in this study amount to 10 (obtained from 11-1). The degrees of freedom are still divided into two, namely the degree of freedom of the numerator (df1) and the degree of freedom of the denominator (df2). The denominator df (df2) is calculated from df minus the quantifier df (df1). Because only one independent variable is used here (*IGF-1* gene polymorphism point), the numerator df is 1 and the denominator df is 9. Using the F-distribution table for a 0.05 or 95% confidence level, the numerator's degrees of freedom (df for Regression) is 1, and the degrees of freedom of the denominator (df for Residual) is 9, the critical value F (Ftable) is 5.1174.

Based on Table 2, the linear regression F test value or f statistic value of the 12 points of polymorphism is 1.067 at the first point, 0.014 at the second point, 1.101 at the third point, 0.218 at the fourth point, 0.239 at the fifth, sixth, and seventh points, 0.136 at the eighth, ninth, and eleventh, 0.042 at the tenth point, and 2.109 at the twelfth point. Because all the statistical values of f are smaller than the critical value of F (5.1174), it can be concluded that the regression model is not statistically significant. According to the Linear Regression F test results, *IGF-1* gene polymorphism did not significantly affect F_5 Golden Kamper chickens. One of the factors that caused the *IGF-1* gene polymorphism did not significantly affect the weight growth of F_5 Golden Kamper chickens was the presence of linkage disequilibrium. Linkage disequilibrium is likely to occur between the polymorphism points found in the *IGF-1* gene and other linked genes, which directly affect the weight growth of F_5 Golden Kamper chickens. Inbreeding or crossbreeding can increase the covariance (relationship between random variables) between alleles at different loci so that it can be a factor in the occurrence of linkage disequilibrium [13].

4 Conclusion

This study reveals that the weight gain in F5 GK chickens over 7week was comparatively lower than that observed in F4 Golden Kamper, Pelung, and layer chickens. However, it surpassed the weight gain observed in F2 GK. Polymorphism of the Insulin-like Growth Factor-1 gene 5'UTR region in F_5 Golden Kamper chickens did not correlate or significantly influence ($p>0.05$) the growth of chicken weight.

We are grateful to the editor and reviewers for their valuable comments and suggestions, which resulted in an improved manuscript. We are also grateful to the Final Project Recognition Grant Gadjah Mada University Number 5075/UNI.P.II/Dit-Lit/PT.01.01/2023 that have funded this research.

References

1. BPS, Statistik Indonesia: Statistical Yearbook of Indonesia 2023 (Badan Pusat Statistik, Indonesia, 2023)
2. I. Lesmana, Asosiasi Polimorfisme Promoter Gen FSHR dengan Perkembangan Folikel Ovarium Ayam Hibrida [*Gallus gallus gallus* (Linnaeus, 1758)] Hasil Persilangan ♀ Ras Petelur dengan ♂ Pelung, (Thesis, Gadjah Mada University, Yogyakarta, 2016)
3. I. W. S. Mahardhika, B. S. Daryono, A. A. C. Dewi, S. N. Hidayat, G. I. Firmansyah, P. S. Setyowati, U. R. Riswanta, M. D. Pratama, Ju. Pet., **17**, 1 (2020)
4. R. Saxena, V. K. Saxena, V. Tripathi, N. A. Mir, K. Dev, J. Begum, R. Agarwal, A. Goel, Gen. Comp. Endocrino., **288** (2020)
5. W. P. B. Putra, S. T. Nugraheni, Y. Irnidayanti, S. Said. J. Ilmu Ternak & Vet., **23**, 174-179 (2018)
6. A. M. Oberbauer, Front. Endocrinol., **4**, 1-9 (2013)
7. S. Al-Hassani, D. H. Al-Hassani, I. A. Abdul-Hassan, Asian J. Poult. Sci., **9**, 213-222 (2015)
8. R. B. Widelitz, G. Lin, Y. Lai, J. A. Mayer, P. Tang, H. Cheng, T. Jiang, C. Chen, C. Chuong, Dev. Growth Differ., 1-15 (2018)
9. B. Song, D. Tang, S. Yan, H. Fan, G. Li, M. S. Shahid, T. Mahmood, Y. Guo, J. Anim. Sci. Biotechnol., **12**, 1, 1-12 (2021)
10. P. A. Dewanata, M. Mushlih, Indo. J. Innov. Stud., **15**, 1-10 (2021)
11. E. I. Obilor, E. C. Amadi, Int. J. Inno. Maths Statistics & Energy Policies, **6**, 1, 11-23 (2018)
12. F. Jabnabillah, N. Margina, J. Sint., **1**, 1, 14-18 (2022)
13. M. Amills, N. Jimenez, D. Villalba, M. Tor, E. Molina, D. Cubilo, C. Marcos, A. Francesch, A. Sanchez, J. Estany, Poult. Sci., **82**, 10, 1485-1493 (2003)