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Genetic diversity of transforming growth factor beta 3 (*TGF-β3*) gene in Kedu chickens

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ABSTRACT

The aim of this study was to identify the genetic diversity of *TGF-β3* gene in Kedu chickens. The materials used consisted of 10 Kedu chickens derived from different maternal parents. These methods included blood collection, DNA extraction, gene amplification, sequencing, and data analysis. The parameters analyzed were the number of segregating sites, types of mutations, amino acid alteration, allele frequencies, genotype frequencies, Hardy-Weinberg Equilibrium (HWE), and heterozygosity values. Data analysis was performed using MEGA12 and DNASP v.6. The results showed that there were four mutation points in *TGF-β3* gene in Kedu chickens, namely at sites 26, 89, 191, and 214. The HWE values for mutation point at sites 26 and 191 were 0.311, whereas those mutation points at sites 89 and 214 were 0.028, indicating a deviation from equiblirium. The average heterozygosity value in this study was 0.175, which was considered low. An amino acid alteration was observed at site 214, where Serine was changed to arginine. In conclusion, Kedu chicken population exhibits low genetic diversity based on the *TGF-β3* gene, as indicated by the number of segregating sites, genotype and allele frequencies, HWE values, and heterozygosity values. The mutation point at site 214 results in an amino acid substitution and has the potential to serve as a candidate genetic marker for the selection of carcass production traits.

Introduction

As Indonesia's population continues to grow, the demand for protein is increasing. Poultry farming accounts for approximately 60% of the nation's animal protein supply, particularly through chickens, including local breeds. One of the most popular local chicken breeds among communities is Kedu chicken. The Kedu chicken is an indigenous breed from Central Java that is favored by traditional farmers due to its unique characteristics, such as jet-black feathers, yellow legs, and tender meat. Originating from Kedu Village in Temanggung, Central Java, the Kedu chicken is considered part of Indonesia's valuable genetic resources (Ustadha *et al*., 2016). It is recognized as a dual-purpose breed, appreciated for both meat and egg production. Its advantages include higher productivity compared to other local breeds, a distinctive meat flavor, and significant cultural value within local communities (Baharudin *et al*., 2019).

Broiler farming relies heavily on meat production to meet the growing consumer demand for high-quality animal protein. In addition to environmental influences, genetic factors play a crucial role in determining carcass production in livestock. Genetics significantly affects production traits and carcass quality, including the percentage of boneless meat, fat layer thickness, and carcass size. In chickens, carcass yield is influenced not only by genetic factors but also by final body weight, feed composition, and environmental conditions (Nurhidayat *et al*., 2020). This is because livestock productivity cannot exceed its genetic potential, even under optimal feed and management conditions. This highlights the importance of implementing breeding programs through genetic selection, particularly for improving carcass production traits in Kedu chickens.

The Transforming Growth Factor Beta 3 (*TGF-β3*) gene plays a vital role in various biological processes in chickens, including growth, connective tissue development, and immune response. *TGF-β3* is a member of the receptor family and is involved in the regulation of growth and development in poultry (Wu *et al*., 2020). The *TGF-β3* gene is located on chromosome 5, spans 13,997 bp, and comprises 7 exons and 6 introns (Accession ID: 396438). This gene holds significant potential as a candidate genetic marker for carcass traits in chickens and may support selection and breeding programs for Kedu chickens. Furthermore, *TGF-β3* has been previously identified as a potential marker for growth traits in broiler chickens (Hosnedlova *et al*., 2020).

Based on the reasons mentioned above, this study aimed to identify the genetic diversity of the *TGF-β3* gene in Kedu chickens. The significance of this study lies in its potential to provide information on genetic markers associated with carcass production, which may support selection and breeding programs to improve carcass quality in Kedu chickens.

Materials and methods

Blood collection and DNA extraction

Blood samples from 30-day-old male Kedu black chickens with red combs were collected in a volume of 3 ml via the brachial vein, located in the wing area. The area was then disinfected using cotton soaked in alcohol. Blood was drawn using a 3 mL syringe and stored in a vacuum tube containing EDTA to prevent coagulation and contamination. The EDTA tubes were placed in a cooling box filled with ice gel to maintain a low temperature.

DNA extraction was carried out using the gSYNC™ DNA Extraction Kit, following the protocol for poultry blood samples. Electrophoresis was conducted on a 1% agarose gel to confirm the presence of successfully extracted DNA. The gel was then visualized using a gel documentation system to verify the results.

Gene amplification, electrophoresis and sequencing

Amplification of the *TGF-β3* gene was carried out using PCR method

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by Benchmark TC 9639, with the extracted DNA serving as the template. The PCR mixture consisted of 3 µL DNA template, 25 µL MyTaq HS Red Mix, 1 µL each of forward (5′-TCAGGGCAGGTAGAGGGTGT-3′) and reverse (5′-GCCACTGGCAGGATTCTCAC-3′) primers (Hosnedlova *et al*., 2020), and 20 µL PCR-grade water, all placed in a 50 µL PCR tube. The resulting PCR product was 294 bp in length, covering partial intron 4, exon 5 and partial intron 5. The PCR products were then electrophoresed on a 1% agarose gel.

Electrophoresis was performed following DNA extraction and PCR amplification to visualize the results. A total of 3µl of sample (gDNA or amplicon) is mixed with 1µl of Loading Dye and loaded into the wells of a 1% agarose gel using a micropipette. The DNA ladder was placed into the final well after the samples. Electrophoresis was carried out at 100 volts for 20 minutes. The results were visualized under UV light using a Gel Documentation System (GDS).

DNA sequencing in this study was conducted to determine the DNA sequence of Kedu chickens that encode the *TGF-β3* gene. Sequencing was carried out using the Sanger method by a private sequencing service (1st Base, Singapore).

Data analysis

Sequencing data were analyzed using Molecular Evolutionary Genetics Analysis version 12 (MEGA 12) to identify mutation points and amino acid changes (Tamura *et al*., 2021). Genetic diversity analysis was conducted using DnaSP version 6 (Rozas *et al*., 2017). Nucleotide alignment of the *TGF-β3* gene was performed using the ClustalW program (Thompson *et al*., 1994). Allele frequencies were calculated as follows:

$$FAi = (\sum Allele\ Ai)/(\sum Allele\ Ai + " \sum Allele\ Bi + ... + \sum Allele\ Ni)$$

where FAi represents the frequency of allele A at the ith locus (Warwick *et al*., 1995). The mean heterozygosity was calculated using the following formula:

$$H = (1 - \sum i^m q^2)/r$$

where *q* represents the allele frequency at each locus, *m* represents the number of alleles, and r represents the number of loci (Nei, 1987). The expected genotype frequencies were calculated based on the Hardy-Weinberg Equilibrium (HWE) theory using the following equations:

$$p^2 + 2pq + q^2 = 1 \text{ (for 2 alleles)}$$

$$p^2 + 2pq + 2pr + q^2 + 2qr + r2 = 1 \text{ (for 3 alleles)}$$

where *p* represents the frequency of the first allele, q represents the frequency of the second allele, and *r* represents the frequency of the third allele (Falconer and Mackay 1996). HWE was assessed using the chi-square (χ^2) test, which was applied to compare the observed and expected heterozygosity values:

$$X^2 = \sum i^k\text{-}1(oi - ei)^2/ei$$

where (χ^2) represents the chi-square value, oi represents the observed genotype frequency, and ei represents the expected genotype frequency (Hartl and Clark, 1997).

Results

Based on the results of PCR, amplification of the *TGF-β3* gene in Kedu chickens was successfully achieved, with an estimated fragment length of approximately 300 bp (Fig. 1), consistent with the expected 294 bp product from primer BLAST analysis. Electrophoresis visualization showed that samples F, G, H, I, and J produced clear, bright, and thick DNA bands, while samples A, B, C, D, and E exhibited faint and thin bands. Sanger sequencing was performed, and the diversity of the *TGF-β3* gene is summarized in Table 1. The PCR product was 294 bp in length. After trimming, 263 bp of sequence data were used for analysis. Alignment of ten Kedu chicken sequences revealed four mutation points at positions 26, 89, 191, and 214. These included two singleton variable sites (positions 89 and 214) and two parsimony-informative sites (positions 26 and 191), as shown in Table 2. Mutations at site 26 (G→A) resulted in genotypes GG (Samples B, D, F, G, H, I, J) and GA (Samples A, C, E); AA was not observed. At site 89 (C→A), genotypes were CC (Samples A, B, D, E, F, G, H, I, J) and CA (Sample C); AA was absent. At site 191 (C→T), genotypes included CC (Samples B, D, F, G, H, I, J) and CT (Samples A, C, E); TT was not detected. At site 214 (C→A), CC (Samples A, C, D, E, F, G, H, I, J) and CA (Sample B) were found; AA was not present. Two types of nucleotide substitutions were identified: transition mutations (G→A at site 26 and C→T at site 191) and transversion mutations (C→A at sites 89 and 214). At each mutation site, only two genotypes—one homozygous and one heterozygous—were observed. No third genotype was present at any locus.

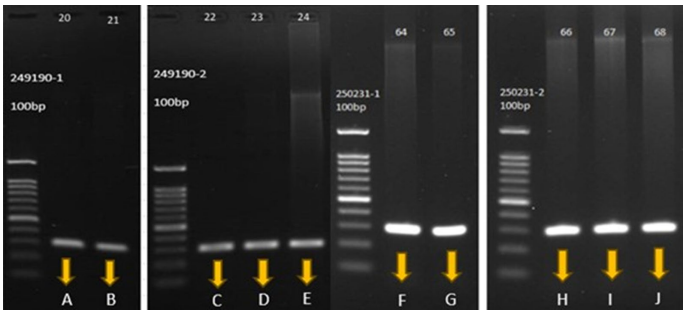


Fig. 1. Visualization of PCR results gen TGF- β3 using agarose gel electrophoresis

Table 1. Genetic Diversity of the *TGF-β3* Gene in Kedu Chickens.

Gene	PCR Product Length*	Analyzed Sequence Length	S	Si	Pa	Mutation Point Location	
						Si	Pa
TGF- β3	294 bp	263 bp	4	2	2	89, 214	26, 191

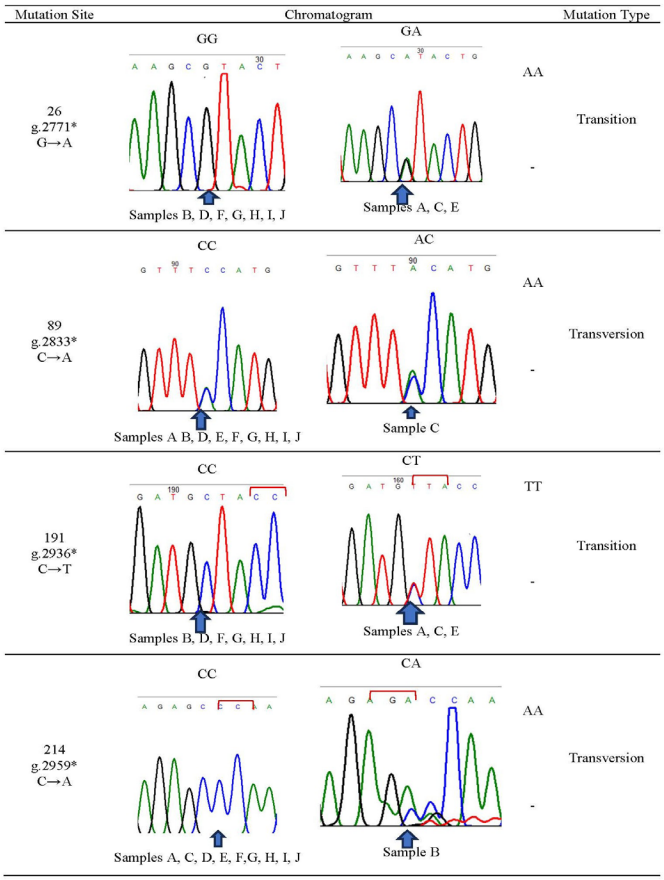
*: The length of the simulated gene sequence based on primer blast, S: Segregating sites, Si: Singleton, Pa: *Parsimony*

The analysis showed that at the 26th mutation site, there were two alleles, G and A. The frequency of the G allele was 0.85, higher than the A allele at 0.15. At the 89th mutation site, the two alleles were C and A, with the C allele having a higher frequency (0.95) compared to the A allele (0.05). At mutation site 191, the C allele frequency was 0.85 and the T allele was 0.15. At site 214, the C allele was dominant at 0.95, while the A allele was 0.05. The allele frequency data for the *TGF-β3* gene in Kedu chickens is presented in Table 3. Genetic diversity in the Kedu chicken population was evaluated based on heterozygosity values and Hardy-Weinberg Equilibrium (HWE), determined using the chi-square test. The genotype frequencies, chi-square values, and heterozygosity values for the *TGF-β3* gene are presented in Table 4. The average heterozygosity (H) was 17.5%, with the highest individual heterozygosity values observed at mutation points 26 and 191. The HWE value of the *TGF-β3* gene in Kedu chickens was lower than the HWE values reported in previous studies.

Based on the analysis, mutations at sites 26 and 89 were located in intron 4 of the *TGF-β3* gene. Since the intron region is not translated into a protein, mutations at these positions did not result in amino acid changes. Mutations at sites 191 and 214 were located in exon 5. The mutation at site 191 was identified as a silent mutation, where the codon change (from CTA to TTA) did not alter the resulting amino acid (leucine). In contrast, the mutation at site 214 was a missense (non-synonymous) mutation that led to an amino acid change. Specifically, the codon AGC (coding for serine) was altered to AGA (coding for arginine) in sample B. This change indicates a potential impact on the structure and function of the protein encoded by the *TGF-β3* gene. The mutation at site 214, there-

fore, has the potential to cause variation in the expressed protein among Kedu chicken samples.

Table 2. Types and Positions of Mutations in the *TGF-β3* Gene



*: Sequence based on GenBank (Accession number: X60091)

Table 3. Allele Frequency of the *TGF-β3* Gene

Mutation Site	Allele	Frequency
26	G	0,85
g.2771* G>A	A	0,15
89	C	0,95
g.2833* C>A	A	0,05
191	C	0,85
g.2936* C>T	T	0,15
214	C	0,95
g.2959* C>A	A	0,05

*: Sequence based on GenBank (Accession number: X60091)

Discussion

The successful amplification of the *TGF-β3* gene fragment (~300 bp) using PCR and its match with the predicted 294 bp from primer BLAST confirms the specificity of the primers used. The use of Sanger sequencing was appropriate for this fragment size, as the method is reliable for detecting nucleotide base mutations within sequences up to 1000 bp (Gomes and Korf, 2018). The clarity of DNA bands in samples F to J indicates high DNA quality and quantity, as described by Yulianti *et al.* (2024). Conversely, faint and thin bands in samples A to E likely reflect low DNA concentration (Herman *et al.*, 2018). The sequencing success was likely influenced by multiple factors including primer quality, amplicon length, and concentration (Crossley *et al.*, 2020).

The identification of four mutation points—including both singleton variable sites and parsimony-informative sites—suggests moderate sequence variability in the *TGF-β3* gene. According to Panigrahi *et al.* (2025), segregating sites indicate nucleotide base variation, while Persada *et al.* (2021) define singleton variable sites as variants that appear only once in the sample set. Positions 89 and 214 matched this description. Positions 26 and 191, where variation occurred in more than one sample, meet the criteria for parsimony-informative sites (Warseno *et al.*, 2022). Transition mutations identified in this study (G→A and C→T) reflect purine-to-purine or pyrimidine-to-pyrimidine changes, in line with Wulandari (2019), while transversion mutations (C→A) represent purine-to-pyrimidine substitutions (Syaikhullah *et al.*, 2017).

The absence of one genotype at each mutation site suggests a deviation from Hardy-Weinberg equilibrium, possibly due to selective pressures. According to Afriani *et al.* (2022a), genotype frequency imbalances may result from selection, mutation, migration, or genetic drift. In this study, artificial selection in livestock production systems such as that involving Kedu chickens may have contributed to the loss of certain genotypes. Breeding preferences for superior traits likely led to the elimination of less favorable genotypes. Furthermore, genetic drift due to reduced population size, inbreeding, or disease could also explain the absence of homozygous minor genotypes, as suggested by Schmenger *et al.* (2022), who noted that such genotypes may be eliminated if they confer lower fitness or survivability.

The observed allele frequencies showed that at all four mutation sites (26, 89, 191, and 214), one allele was consistently more dominant, while the other was minor. These findings differ from those of previous studies. For instance, Hosnedlova *et al.* (2020) reported that Hubbard F15 chickens had equal allele frequencies for C and A (0.5 each), while Cobb E chickens had C (0.6489) and A (0.3511) at the *TGF-β3* locus, based on SNP g.2833 which is equivalent to the mutation at site 89 in present study. Khaerunnisa and Sumantri (2020) also found variation in allele frequencies: in native and Cobb chickens, the frequencies were C (0.42) and A (0.588), and C (0.65) and A (0.35), respectively. In crossbred native ×

Table 4. Genotype Frequency, Chi Square, and Heterozygosity of the *TGF-β3* Gene.

Mutation site	Genotype	Freq.	Obs.	Exp.	HWE	h	H
26 g.2771* G>A	GG	0,7	7	7,225	$X^2 = 0,311$	0,255	0,175
	GA	0,3	3	2,55			
	AA	0	0	0,225			
89 g.2833* C>A	CC	0,9	9	9,025	$X^2 = 0,028$	0,095	
	CA	0,1	1	0,95			
	AA	0	0	0,025			
191 g.2936* C>T	CC	0,7	7	7,225	$X^2 = 0,311$	0,255	
	CT	0,3	3	2,55			
	TT	0	0	0,225			
214 g.2959* C>A	CC	0,9	9	9,025	$X^2 = 0,028$	0,095	
	CA	0,1	1	0,95			
	AA	0	0	0,025			

h: individual heterozygosity, H: average heterozygosity, Freq: genotype frequency, Obs: number of individuals observed, Exp: number of individuals expected, HWE: Hardy-Weinberg Equilibrium, *: sequence based on GenBank (Accession number: X60091).

Cobb chickens (F1), frequencies were C (0.40) and A (0.60), and in F1 × F1 crosses, they were C (0.35) and A (0.65). The difference between this study and prior findings may be due to the limited number of genotypes observed in the current Kedu chicken population, which consisted of only two genotypes per locus. This suggests the presence of strong selection pressures acting on the population.

The presence of dominant and minor alleles, along with the absence of certain homozygous genotypes such as AA at g.2771, g.2833, and g.2959 or TT at g.2936, is consistent with the effects of genetic drift. According to Fauziah *et al*. (2025), genetic drift refers to random fluctuations in allele frequencies, which may lead to the disappearance of specific genetic variants and a reduction in overall genetic variation. This aligns with the view of Lenart *et al*. (2022), who emphasized that genetic drift is a macro-evolutionary process that can cause allele fixation and the loss of alternative alleles across generations.

The allele frequencies observed in this study also indicated that the *TGF-β3* gene in Kedu chickens is polymorphic. This is supported by Afriani *et al*. (2022a), who stated that an allele is considered polymorphic if its frequency is less than 0.99. Moreover, allele frequency is influenced by several evolutionary forces, including selection (both natural and artificial), mutation, migration, genetic drift, and random mating. Fauziah *et al*. (2025) further explained that high allele frequencies typically result from adaptive advantages due to natural selection, while low frequencies may reflect poor survival or reproductive capacity associated with certain alleles.

The use of the chi-square test in this study to assess Hardy-Weinberg equilibrium is consistent with Afriani *et al*. (2022b), who stated that the equilibrium and deviation of data based on Hardy-Weinberg expectations can be evaluated using chi-square analysis. The lower HWE value observed in Kedu chickens compared to other breeds may indicate a deviation from Hardy-Weinberg equilibrium. Hosnedlova *et al*. (2020) reported HWE values of 0.1639 in Hubbard F15 chickens and 1.00 in Cobb E chickens. Such deviations in the Kedu chicken population could be influenced by factors such as artificial selection, genetic drift, inbreeding, or population substructure. The heterozygosity value of 17.5% reflects a relatively low level of genetic diversity in the *TGF-β3* gene. High heterozygosity generally suggests greater genetic variation, providing more opportunities for natural or artificial selection to act on beneficial traits. This aligns with the statement by Munthe *et al*. (2024), who emphasized that greater genetic diversity enhances the potential for selecting superior genotypes. Conversely, lower heterozygosity implies that the environment may play a more significant role than genetic variation in shaping traits. The low heterozygosity values observed here suggest limited genetic variability, which can restrict the adaptive potential and effectiveness of selection within the population. Therefore, factors such as sample size, breed characteristics, and population structure, as noted by Nugroho *et al*. (2016), must be considered in conservation and breeding strategies aimed at maintaining or increasing genetic diversity.

Mutations located in intron 4 at sites 26 and 89 do not contribute to protein variation because introns are not translated during the protein synthesis process. This finding is consistent with the explanation by Agoes *et al*. (2016), who stated that during translation, only exon regions are transcribed and translated into amino acids, whereas intronic regions are removed during mRNA processing. The mutation at site 191, although located in exon 5, was a silent mutation. This means that despite a nucleotide substitution (CTA to TTA), both codons encode the same amino acid, leucine. This type of mutation is explained by Nandariyah *et al*. (2023) as a change that does not alter the resulting protein due to the degeneracy of the genetic code, where different codons can code for the same amino acid.

Conversely, the mutation at site 214 was a missense mutation, which does result in a change in the amino acid sequence—from serine (AGC) to arginine (AGA). This finding is supported by Rahayuningsih *et al*. (2016), who describes missense mutations as genetic alterations in which one amino acid is replaced by another, potentially affecting the resulting protein. The chemical nature of this substitution is notable: serine is an uncharged polar amino acid, while arginine is positively charged and polar. This substitution may influence the hydrogen bonding capability, protein folding, or protein–protein interactions, potentially altering the biological function of the *TGF-β3* protein. This interpretation is aligned with Stryer *et al*. (2002), who emphasized the structural and functional impact of amino acid changes on proteins. Therefore, the mutation at site 214 could play a significant role in functional variation of the *TGF-β3* protein in Kedu chickens. This supports the potential of *TGF-β3* as a candidate genetic marker for traits such as carcass quality or production performance in local chicken breeds.

Conclusion

Kedu chicken population exhibits low genetic diversity, as reflected by the limited number of segregating sites, the predominance of two genotype types per locus, skewed allele frequencies, deviation from Hardy-Weinberg Equilibrium, and low heterozygosity values based on the *TGF-β3* gene analysis. Four mutation sites were identified in the *TGF-β3* gene (positions 26, 89, 191, and 214), with the mutation at position 214 resulting in an amino acid substitution. This non-synonymous mutation may influence the structure and function of the encoded protein, highlighting its potential as a candidate genetic marker for carcass trait selection in Kedu chicken breeding programs. These findings underscore the importance of genetic monitoring and conservation strategies to maintain and improve the genetic resources of indigenous chicken populations.

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Conflict of interest

The authors have no conflict of interest to declare.

References

Afriani, T., Purwati, E., Hellyward, J., Jaswandi, J., Mundana, M., Farhana, A., Rastosari, A., 2022a. Identification of Single Nucleotide Polymorphism (SNP) in early exon 10 of Follicle Stimulating Hormone Receptor (FSHR) gene in Pesisir Cattle. JIPT. 10, 264-276.

Afriani, T., Purwati, E., Hellyward, J., Jaswandi, J., Yurnalis, J., Mundana, Y., Mundana, M., Farhana, A., Rastosari, A., 2022b. Identifikasi keragaman gen FSH bagian ekson 2 menggunakan enzim restriksi TasI pada sapi Pesisir. J. Peternak. Indonesia. 19, 86-95.

Agoes, S., Solihah, B., Pakpahan, A., 2016. Analisis kinerja model pengontrol ekson DNA menggunakan metode model Hidden Markov. Setrum: SETRUM. 3, 69-72.

Baharudin, M., Kurnianto, E., Kismiati, S., 2019. Pengaruh umur induk dan lama penyimpanan terhadap kualitas internal telur ayam Kedu jengger hitam. J. Peternak. Indonesia. 21, 192-197.

Crossley, B. M., Bai, J., Glaser, A., Maes, R., Porter, E., Killian, M., Clement, L., Kurth, T., 2020. Guidelines for sanger sequencing and molecular assay monitoring. J. Ver. Diagn. Invest. 32, 767-775.

Fauziah, S.G., Hilmia, N., Mayasari, N., 2025. Identifikasi keragaman gen Serol Regulatory Element-Binding Protein 1 (SREBP -1) sebagai kandidat gen untuk kualitas daging sapi Pasundan. J. Teknologi Hasil Peternakan. 6, 116-128.

Falconer, D., Mackay T.T., 1996. Introduction to Quantitative Genetics. Pearson Education Limited, Harlow, UK.

Gomes, A., Korf, B., 2018. Genetic Testing Techniques. RobinNH, Farmer MB (Editors). In Pediatrics Cancer Genetics, pp 47-64.

Hartl, D.L., Clark, A.G., 1997. Principles of Population Genetics 3rd Ed. Sinauer Associates Inc, Sutherland.

Herman, H., Nainggolan, M., Roslim, D.I., 2018. Optimasi suhu annealing untuk empat primer RAPD pada kacang hijau (*Vigna radiata* L.). Dinamika Pertanian. 34, 41-46.

Hosnedlova, B., Vernerova, K., Kizek, R., Bozzi, R., Kadlec, R., Curn, V., Kouba, F., Fernandez, C., Machander, V., Horna, H., 2020. Associations between IGF1, IGFBP2 and TGFß3 genes polymorphisms and growth performance of broiler chicken

lines. Animals. 10, 1- 24.
Khaerunnisa, I., Sumantri, C., 2020. The *TGF-β3* gene polymorphisms and carcass components in Kampung x meat type chicken cross. J. I. Produksi Teknol. Hasil Peternakan. 8, 42-47.
Lenart, P., Bienertová-Vašků, J., Berec, L., 2022. Learning mitigates genetic drift. Sci. Rep. 12, 1-10.
Munthe, R., Ardian, A., Setiawan, K., Sa'diyah, N., 2024. Keragaman genetik dan heritabilitas karakter tinggi tanaman dan jumlah daun beberapa genotipe sorgum (*Sorghum bicolor* L. Moench). J. Agrotek Tropika. 2, 90-96.
Nandariyah, N., Prastyaningrum, S., Manurung, I.R., Sutarno, S., Riyatun, R., 2023. Karakterisasi morfologi galur harapan M0 dan M3 padi hitam hasil iradiasi sinar gamma. J. Penelitian Agronomi. 25, 70-77.
Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.
Nugroho, B. P. S., Sutopo, S., Kurnianto, E., 2016. Polimorfisme protein darah ayam Kedu jengger merah dan jengger hitam di Satuan Kerja Non Ruminansia Temanggung. JITPI. 2, 159-165.
Nurhidayat, F., Mahfudz, L.D., Sunarti, D., 2020. Efek perbedaan pataran terhadap produksi karkas ayam broiler yang dipelihara di kandang closed house. J. Sain Peternakan Indonesia 15, 406-413.
Panigrahi, S.K., Patro, S.P., Agrawal, S., Panda, P., Parida, S.R., Mohanty, P., Das, J., Behera, R. K., Nayak, N.R., 2025. Meta-Analysis of population genetics of *Aedes vittatus* in India based on COI gene: A first report from a public health point of view. South East. Eur. J. Public Health 26, 1756-1763.
Persada, A.P., Solihin, D.D., Affandi, R., 2021. Genetic characteristic of giant featherback, *Chitala lopis* (Bleeker, 1851) from Lampung and Kalimantan using COI Gene. J. Iktiologi Indones. 21, 49-60.
Rahayuningsih, S.E., Hamanoue, H., Matsumoto, N. 2016. Peran mutasi gen CRELD1 pada efek septum ventrikel dan hubungannya dengan manifestasi klinis. Sari Pediatri. 10, 225-229.
Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Mol. Biol. Evol. 34, 3299-3302.
Schmenger, T., Diwan, G.D., Singh, G., Apic, G., Russell, R.B., 2022. Never-homozygous genetic variants in healthy populations are potential recessive disease candidates. NPJ Genom. Med. 7, 54.
Stryer, L., Berg, J.M., Tymoczko, J.L., 2002. Biochemistry (5th ed.). W.H. Freeman and Company.
Syaikhullah, G., Darwati, S., Sumantri, C., 2017. Asosiasi keragaman Growth Hormone Secretagogue Receptor (GHSR Hin6i) terhadap bobot karkas ayam Kampung pada umur 12 minggu. J. I. Produksi Teknol. Hasil Peternakan. 5, 117-121.
Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022-3027.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673-4680.
Ustadha, C., Sutopo, Sumeidiana K., I., 2016. Pengaruh perbedaan bobot telur terhadap bobot tetas dan mortalitas ayam Kedu jengger merah dan ayam Kedu jengger hitam. J. Agro. 34, 65-71
Warseno, T., Efendi, M., Chasani, A.R., Daryono, B.S., 2022. Genetic variability and phylogenetic relationships of *Begonia multangula* based on ATPB-RBCL non-coding spacer of CPDNA sequences. Biodiversitas. 23, 5491-5501.
Warwick, E.J., Astuti, J.M., Hardjosoebroto, W., 1995. Pemuliaan Ternak. Edisi ke-5. Gadjah Mada University Press, Yogyakarta.
Wu, P., Zhang, X., Zhang, G., Chen, F., He, M., Zhang, T., Wang, J., Xie, K., Dai, G., 2020. Transcriptome for the breast muscle of Jinghai yellow chicken at early growth stages. PeerJ. 8: e8950.
Wulandari, A., 2019. Filogeni beberapa sapi lokal indonesia menggunakan DNA mitokondria COI (Cytochrome Oxidase Sub unit I). JITPT. 6, 278-282.
Yulianti, S., Islamidan, R.Z., Pramudyawardani, E.F., 2024. Analisis konsentrasi dan kemurnian Deoxyribo Nucleic Acid (DNA) dari rumput pakan ternak. AgriVet. 12, 167-179.