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Association analysis of the transforming growth factor-β2 gene with growth performance in Kedu chickens

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ABSTRACT

The Transforming Growth Factor-β2 (TGF-β2) gene is one of the cytokine genes that plays an important role in regulating growth processes and disease resistance. This study aimed to analyze the association between the TGF-β2 gene and growth performance in Kedu chickens. A total of 130 Kedu chickens, consisting of 72 males and 58 females, were observed for 10 weeks. Growth parameters measured included body weight, feed intake, body weight gain, and feed conversion ratio. Identification of the single nucleotide polymorphism (SNP) in the TGF-β2 gene was performed using the PCR-RFLP method. The association analysis between the g.640 T>C SNP polymorphism of the TGF-β2 gene and growth performance was conducted using analysis of variance under a General Linear Model (GLM). The results showed that the g.640 T>C SNP of the TGF-β2 gene was polymorphic and not in Hardy–Weinberg equilibrium. The TT genotype exhibited better growth performance (body weight gain, feed intake, and feed conversion ratio) than the CC genotype, particularly at 10 weeks of age in both female and unsexed chickens.

Keywords: body weight gain, feed intake, feed conversion ratio, growth performance, Kedu chicken, TGF-β2 gene.

INTRODUCTION

Indonesian native chickens possess diverse genetic potential with several advantages such as the ability to utilize low-quality feed, superior meat flavor preferred by consumers, high adaptability to tropical environments, and good tolerance to diseases (Pagala *et al.,* 2017; Albani *et al.,* 2025). One of the native chicken breeds exhibiting these characteristics is the Kedu chicken, which originates from Central Java Province (Sutopo *et al.*, 2022).

Despite its advantages, the population and genetic potential of Kedu chickens have declined in line with the rapid development of the commercial poultry industry. The dominance of commercial broiler chickens in the market has reduced the demand for local breeds, as Kedu chickens still have relatively lower growth rates and productivity compared to commercial lines

(Setiaji *et al.*, 2025). Therefore, improving the genetic quality of Kedu chickens is necessary, particularly through molecular approaches to identify and utilize superior genes associated with enhanced production performance.

One of the genes that plays an important role in regulating growth traits in chickens is the Transforming Growth Factor Beta 2 (TGF-β2) gene (Tang *et al.*, 2010). This gene belongs to the Transforming Growth Factor Beta (TGF-β) superfamily, which is primarily involved in cell differentiation, muscle tissue development, and growth regulation (Li *et al.*, 2003; Tang *et al.*, 2010). The researchers Li *et al.* (2003) successfully identified the TGF-β2 gene in broiler and Leghorn chickens. The identification was carried out in exon 1, with a PCR product length of 284 bp, using the RsaI restriction enzyme, indicating the presence of genetic variation that could be associated with differences in growth and immune response between chicken breeds. The study by Tang *et al.* (2010) revealed that the TGF-β2 gene is polymorphic, with a Single Nucleotide Polymorphism (SNP) located at position 640 (T>C), resulting in two alleles (T and C) and three genotypes: TT, TC, and CC.

In several populations of native chickens from different countries, such as indigenous chickens from China, Malaysia, Indonesia (Sentul and Tolaki), and Iraq, the TGF-β2 gene at the same fragment has been reported to be polymorphic (Tang *et al.*, 2010; Tohidi *et al.*, 2012; Muhsinin *et al.*, 2017; Sahib *et al.*, 2021). Studies on the SNP g.640T>C in the TGF-β2 gene have shown significant associations with growth performance, particularly body weight, body weight gain, feed intake, and feed conversion ratio (Sahib *et al.*, 2021), as well as with breast muscle weight and carcass percentage (Niarami *et al.*, 2014).

Based on the biological role of the TGF-β2 gene in growth regulation, this study aimed to analyze the association between TGF-β2 gene polymorphism and growth performance in Kedu chickens. The identification of genetic markers associated with growth performance is expected to provide a foundation for developing breeding programs to improve the productivity of Indonesian native chickens.

MATERIALS AND METHODS

Ethical Approval

The experimental protocol and animal treatment in this study were approved by the Animal Research Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. 61-08/A-18/KEP-FPP).

Animal Husbandry

This study was conducted for 10 weeks at the Poultry House, Faculty of Animal and Agricultural Sciences, Diponegoro University. Genetic analyses were performed at the Laboratory of Genetics, Breeding, and Reproduction, Faculty of Animal and Agricultural Sciences, Diponegoro University.

The experimental animals consisted of 130 Kedu chickens, comprising 72 males and 58 females, obtained from hatched eggs. The hatching eggs were collected from Kedu chicken breeders located in Temanggung, Central Java. After hatching, the day-old chicks (DOC) were weighed to determine their initial body weight and were individually identified using leg bands. Weighing was performed after the chicks' feathers were completely dry.

The chickens were reared for 10 weeks. From 4 to 10 weeks of age, they were kept in individual cages measuring approximately 35 × 35 × 40 cm, each labeled with an identification number. The cages were placed in a poultry house measuring 5 × 15 m².

Commercial feed containing 20% crude protein and 2900–3000 kcal/kg metabolizable energy was provided ad libitum. Drinking water was also provided ad libitum. The body weight of each bird was recorded weekly to determine body weight gain during the rearing period. At the end of the 10th week, blood samples were collected from the brachial vein in the wing area. The samples were used for bacterial challenge testing and genotyping analysis. Blood was collected using a 3-mL syringe, with approximately 1–2 mL of blood obtained from each bird.

Growth Performance

The growth performance parameters observed in this study included body weight (g/bird). The individual body weight of each chicken was measured weekly up to 10 weeks of age. Feed intake (g/bird) was calculated daily as the difference between the amount of feed offered

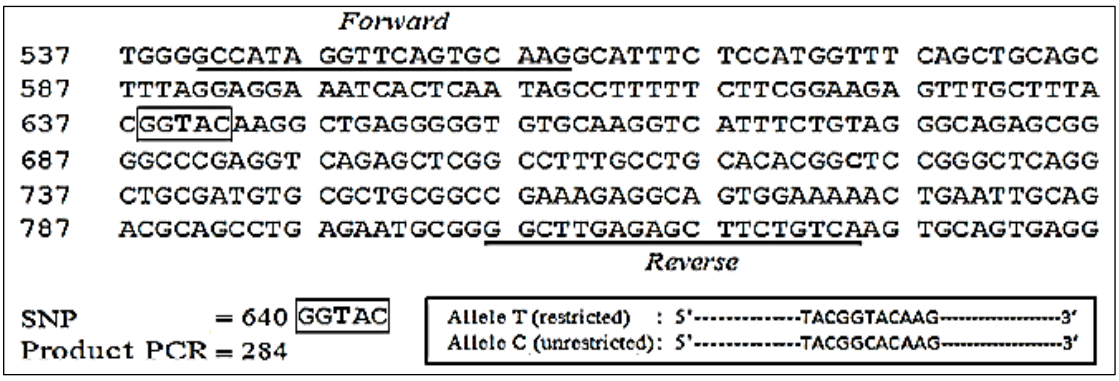

Figure 1. Amplified target region of the TGF-β2 gene. (GenBank: X59081.1)

and the remaining feed. Body weight gain (g/bird/week) was determined by subtracting the initial body weight from the final body weight and dividing the result by the 10-week rearing period. Feed conversion ratio (FCR) was calculated by dividing the average feed intake (g/bird/week) by the average body weight gain (g/bird/week).

DNA Extraction and Amplification of the TGF-β2 Gene

Genomic DNA was extracted from 130 chicken blood samples using the Thermo Scientific GeneJet Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. A pair of specific primers was used to amplify a fragment of the TGF-β2 gene encompassing the region containing the expected single-nucleotide polymorphism (SNP), as previously described by Li *et al.* (2003). The forward primer (F) sequence was 5′-GCC ATA GGT TCA GTG CAA G-3′, and the reverse primer (R) sequence was 5′-TGA CAG AAG CTC TCA AGC C-3′. Based on sequence data available in the National Center for Biotechnology Information (NCBI) database (accession no. X59081.1), the amplified region is located within a partial segment of exon 1 of the TGF-β2 gene, as shown in Figure 1.

Amplification of the TGF-β2 gene fragment was carried out using the Polymerase Chain Reaction (PCR) method. The PCR reaction mixture had a total volume of 50 µL, consisting of 3 µL of DNA template, 20 µL of Taq polymerase master mix (Genoid), 1 µL of forward primer, 1 µL of reverse primer, and 25 µL of nuclease-free water. The reaction mixture was transferred into a 0.2 mL PCR tube, and the amplification process was conducted using a Thermo Scientific thermal cycler (Thermo Fisher Scientific, USA). The PCR amplification program consisted of an initial denaturation at 95°C for 1 min, followed by 39 cycles of denaturation at 95°C for 15 s, annealing at 54°C for 15 s, and extension at 72°C for 10 s. A final extension step was performed at 72°C for 5 min. A 100-bp marker ladder was employed to determine the amplicon length using a UV transilluminator.

Genotyping of the TGF-β2 gene was performed using the PCR-RFLP method with the restriction enzyme RsaI. As much as 10 µL PCR product of the TGF-β2 gene was made as a reaction mixture by adding 1µL RsaI enzyme (10U/µL), 2 µL buffer, and 18 µL water nuclease-free. The solution that had been thoroughly mixed was set for 16 hours at a temperature of 37°C in a dry bath (Claver, UK). The fragments displayed in the electrophoresis results of 2% agarose gel were used to determine the type of genotype.

Genotype and Allele Frequency Analysis

After the genotypes of the TGF-β2 gene were identified using the PCR-RFLP method, genotype and allele frequencies, observed and expected heterozygosity, and Hardy–Weinberg equilibrium values were calculated according to the method described by Nei and Kumar (2000).

Data Analysis

The association between gene diversity and growth performance traits was analyzed using analysis of variance (ANOVA) under the General Linear Model (GLM) procedure (SAS Inst. Inc., Cary, NC, USA). The statistical model applied was:

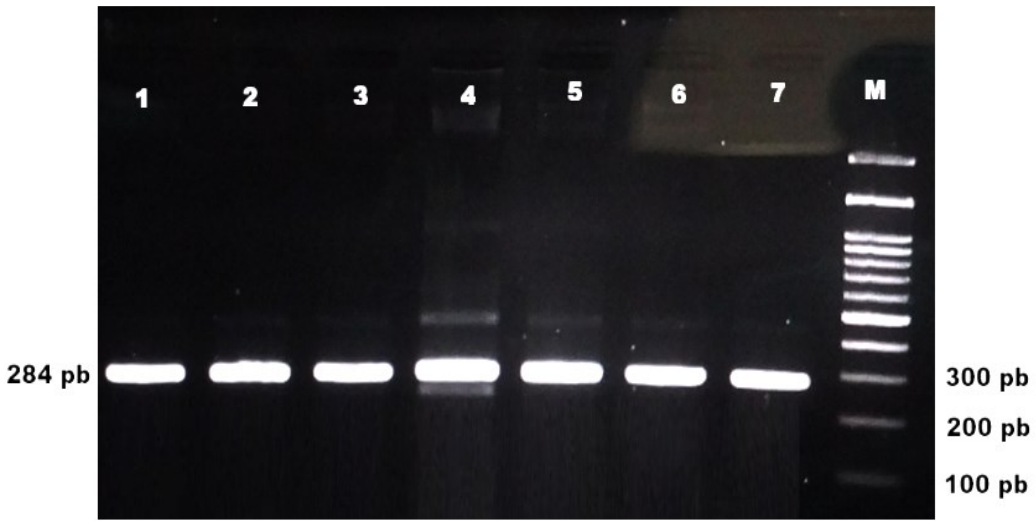

Figure 2. Visualization of the amplified fragment of the TGF-β2 gene in Kedu chickens using 1% agarose gel electrophoresis. (M = 100 bp DNA marker; 1–7 = Kedu chicken DNA samples).

$$y_{ijl} = \mu + G_i + S_j + \varepsilon_{ijl}$$

Where Y_{ijl} represents the observed growth performance traits (body weight, feed intake, body weight gain, or feed conversion ratio), μ is the overall mean, G_i is the fixed effect of genotype, S_j is the fixed effect of sex, and $\mathcal{E}_{ijl}$ is the random error term. Differences were considered statistically significant at P<0.05.

RESULTS AND DISCUSSION

Genotyping of the TGF-β2 Gene

Genotyping of the TGF-β2 gene was performed on exon 1, resulting in a PCR product of 284 bp. The visualization of the PCR amplification product is presented in Figure 2. Digestion of the TGF-β2 gene using the RsaI restriction enzyme successfully identified two alleles, T and C. These two alleles produced three genotypes: TT, TC, and CC (Figure 3). The T allele generated a single band at 284 bp, the C allele produced two bands at 184 bp and 100 bp, while the heterozygous TC genotype displayed three bands at 284 bp, 184 bp, and 100 bp.

Genetic diversity of the TGF-β2 gene

The allele and genotype frequencies of the TGF-β2 gene in Kedu chickens are presented in Table 1. RFLP analysis using the RsaI restriction enzyme on the TGF-β2 gene fragment successfully identified two alleles, T and C. The overall frequency of the T allele in Kedu chickens was 0.55, while that of the C allele was 0.45. These results indicate that the TGF-β2 gene is polymorphic. According to Allendroft *et al.* (2013), a population is considered polymorphic when the frequency of a single allele is less than 0.99.

The genotype distribution of the TGF-β2 gene in Kedu chickens showed that the heterozygous genotype (TC) had the highest frequency (0.75), while the homozygous recessive genotype (CC) had the lowest frequency (0.08). This indicates the dominance of the heterozygous genotype within the observed Kedu chicken population.

Association of the TGF-β2 Gene with Body-Weight

The association analysis of the TGF-β2 gene with body weight in Kedu chickens was conducted to determine the extent to which the gene's genotypes influence the growth performance of Kedu chickens, as presented in Table 2.

The analysis results (Table 2) show that the TGF-β2 gene genotypes are associated with body weight in Kedu chickens at the early growth stage. At 1 week of age, male chickens with TT and TC genotypes had higher body weights compared to those with the CC genotype (P<0.05).

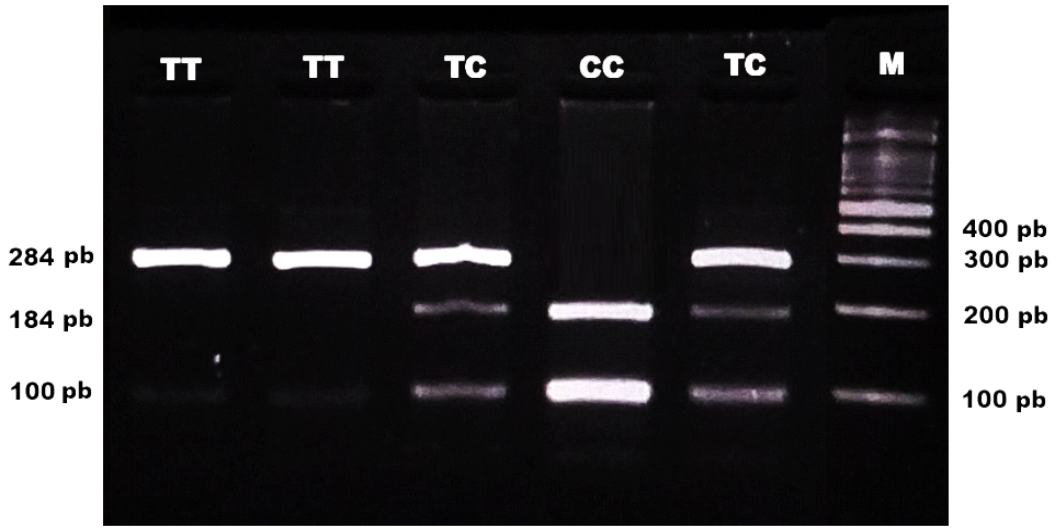

Figure 3. Visualization of PCR-RFLP fragments of the TGF-β2 gene using 2% agarose gel electrophoresis. (M = 100 bp DNA marker; TT, TC, CC = genotypes).

Table 1. Allele and genotype frequencies, heterozygosity, and Hardy–Weinberg equilibrium of the TGF-β2 gene in Kedu chickens

Description	Value
Allele frequency	
T	0.55
C	0.45
Genotype frequency	
TT (n = 22)	0.17
TC (n = 98)	0.75
CC (n = 10)	0.08
Observed heterozygosity (Ho)	0.75
Expected heterozygosity (He)	0.50
χ^2 value (HWE)	35.24

n: Number of samples (total = 130)

This indicates that the T allele may have a positive effect on early growth, possibly through enhanced proliferation and differentiation of muscle cells regulated by the TGF-β2 gene. This gene is known to play an important role in regulating growth, differentiation, and muscle tissue development in poultry (Li *et al.*, 2003; Niarami *et al.*, 2014).

Although no significant differences were observed among genotypes from 2 to 10 weeks of age, the mean values showed that chickens with the TT genotype generally had higher body weights than those with the CC genotype, particularly between 3 and 10 weeks of age. These findings are consistent with Tang *et al.* (2010), who reported that Malaysian native chickens with the TT genotype had higher body weights than those with the CC genotype at 11 weeks of age. Similar results were also reported in Iraqi local chickens aged 11–13 weeks, where the TT genotype exhibited better growth performance than the CC genotype (Sahib *et al.*, 2021).

Association of the TGF-β2 Gene with Feed Intake

The association analysis between the TGF-β2 gene and feed intake was conducted to evaluate whether the gene's genotypes are related to

Table 2. Association of the TGF-β2 Gene with Body Weight (g/bird) of Kedu Chickens Aged 0–10 Weeks

Age (weeks)	Sex	TT (n)	TC (n)	CC (n)	P-value
0	Male	30.36±2.24 (14)	31.44±2.81 (54)	28.75±4.11 (4)	P>0.05
	Female	32.63±3.96 (8)	31.43±2.63 (44)	32.17±4.22 (6)	P>0.05
	Unsexed	31.18±3.10 (22)	31.44±2.72 (98)	30.80±4.32 (10)	P>0.05
1	Male	63.93±5.21[a] (14)	63.67±7.48[a] (54)	56.50±4.65[b] (4)	0.000
	Female	65.25±6.82 (8)	61.18±7.66 (44)	65.50±6.44 (6)	P>0.05
	Unsexed	64.41±5.72 (22)	62.55±7.62 (98)	61.90±7.20 (10)	P>0.05
2	Male	120.64±12.07 (14)	122.04±14.23 (54)	112.50±8.96 (4)	P>0.05
	Female	121.13±9.70 (8)	115.55±13.03 (44)	121.67±13.44 (6)	P>0.05
	Unsexed	120.82±11.03 (22)	119.12±14.02 (98)	118.00±12.23 (10)	P>0.05
3	Male	192.21±23.50 (14)	195.39±21.80 (54)	181.00±19.58 (4)	P>0.05
	Female	188.88±16.23 (8)	176.16±18.19 (44)	184.83±20.41 (6)	P>0.05
	Unsexed	191.00±20.79 (22)	186.76±22.33 (98)	183.30±19.06 (10)	P>0.05
4	Male	308.07±43.40 (14)	297.44±45.53 (54)	291.50±38.80 (4)	P>0.05
	Female	279.38±35.96 (8)	261.52±35.56 (44)	275.83±13.82 (6)	P>0.05
	Unsexed	297.64±42.39 (22)	281.32±44.90 (98)	282.10±25.95 (10)	P>0.05
5	Male	434.64±38.60 (14)	428.24±50.93 (54)	421.25±57.93 (4)	P>0.05
	Female	397.50±44.80 (8)	377.27±39.44 (44)	395.00±25.69 (6)	P>0.05
	Unsexed	421.14±43.89 (22)	405.36±52.50 (98)	405.50±40.86 (10)	P>0.05
6	Male	570.00±56.16 (14)	573.06±55.93 (54)	566.25±59.77 (4)	P>0.05
	Female	519.38±43.87 (8)	484.66±44.14 (44)	510.00±52.35 (6)	P>0.05
	Unsexed	551.59±56.70 (22)	533.37±67.28 (98)	532.50±59.64 (10)	P>0.05
7	Male	715.36±76.50 (14)	719.72±74.48 (54)	705.00±81.34 (4)	P>0.05
	Female	641.88±47.13 (8)	596.36±57.65 (44)	630.83±71.86 (6)	P>0.05
	Unsexed	688.64±75.31 (22)	664.34±91.15 (98)	660.50±80.88 (10)	P>0.05
8	Male	852.86±79.34 (14)	854.54±79.48 (54)	846.25±79.20 (4)	P>0.05
	Female	763.13±42.08 (8)	708.41±70.04 (44)	750.00±90.99 (6)	P>0.05
	Unsexed	820.23±80.24 (22)	788.93±104.71 (98)	788.50±95.72 (10)	P>0.05
9	Male	1035.36±106.62 (14)	1036.48±98.30 (54)	1033.75±96.64 (4)	P>0.05
	Female	913.75±54.82 (8)	844.66±77.87 (44)	885.00±97.47 (6)	P>0.05
	Unsexed	991.14±107.81 (22)	950.36±131.01 (98)	944.50±119.55 (10)	P>0.05
10	Male	1181.07±114.09 (14)	1171.11±102.45 (54)	1166.25±105.70 (4)	P>0.05
	Female	1036.25±56.30 (8)	965.23±85.16 (44)	973.33±121.39 (6)	P>0.05
	Unsexed	1128.41±119.16 (22)	1078.67±139.80 (98)	1050.50±147.77 (10)	P>0.05

n: Number of samples; different superscripts within the same row indicate significant differences (P<0.05).

differences in feed consumption patterns in Kedu chickens, as presented in Table 3.

The analysis results (Table 3) show that, in general, there were no significant differences in feed intake among TGF-β2 genotypes in Kedu chickens at most observation ages. However, at 10 weeks of age, a significant difference (P<0.05) was observed among the TT, TC, and CC genotypes, particularly in female chickens. The TT and TC genotypes exhibited relatively similar feed intake patterns across most periods, whereas the CC genotype tended to have lower feed intake at several ages, especially in females at 10 weeks of age. Female chickens with the CC genotype (464.11 ± 106.26 g/bird/week) had significantly lower feed intake compared to the TT genotype (531.29 ± 40.49 g/bird/week), while the TC genotype (458.98 ± 55.30 g/bird/week) showed intermediate values. A similar trend was observed in the unsexed group, suggesting that

Table 3. Association of the TGF-β2 Gene with Feed Intake (g/bird/week) of Kedu Chickens Aged 4–10 Weeks

Age (weeks)	Sex	TT (n)	TC (n)	CC (n)	P-value
4-5	Male	218.27±44.60 (14)	224.58±42.57 (54)	219.05±34.16 (4)	P>0.05
	Female	202.02±32.32 (8)	199.89±35.39 (44)	201.46±39.00 (6)	P>0.05
	Unsexed	212.36±40.54 (22)	213.49±41.20 (98)	208.49±36.29 (10)	P>0.05
5-6	Male	316.65±68.61 (14)	334.02±68.32 (54)	346.30±73.42 (4)	P>0.05
	Female	286.55±64.79 (8)	249.37±43.02 (44)	262.23±73.48 (6)	P>0.05
	Unsexed	305.71±67.32 (22)	296.02±71.84 (98)	295.86±81.74 (10)	P>0.05
6-7	Male	350.81±65.87 (14)	357.56±54.44 (54)	330.83±77.94 (4)	P>0.05
	Female	323.61±26.70 (8)	282.50±55.92 (44)	318.00±51.25 (6)	P>0.05
	Unsexed	340.92±55.71 (22)	323.86±66.43 (98)	323.13±59.40 (10)	P>0.05
7-8	Male	307.19±60.75 (14)	312.14±82.36 (54)	323.10±58.91 (4)	P>0.05
	Female	287.79±61.48 (8)	271.23±79.97 (44)	274.68±59.47 (6)	P>0.05
	Unsexed	300.13±60.29 (22)	293.77±83.42 (98)	294.05±61.21 (10)	P>0.05
8-9	Male	446.74±84.53 (14)	448.11±90.86 (54)	493.63±28.04 (4)	P>0.05
	Female	399.16±76.41 (8)	352.70±79.31 (44)	350.77±84.49 (6)	P>0.05
	Unsexed	429.44±83.17 (22)	405.27±97.85 (98)	407.91±98.34 (10)	P>0.05
9-10	Male	540.73±42.32 (14)	529.76±73.19 (54)	534.43±54.60 (4)	P>0.05
	Female	531.29±40.49^{a} (8)	458.98±55.30^{a} (44)	417.23±109.28^{b} (6)	0.002
	Unsexed	537.30±40.95^{a} (22)	497.98±74.40^{ab} (98)	464.11±106.26^{b} (10)	0.019

n: Number of samples; different superscripts within the same row indicate significant differences (P<0.05).

Table 4. Association of the TGF-β2 Gene with Body Weight Gain (g/bird/week) of Kedu Chickens Aged 4–10 Weeks

Age (weeks)	Sex	TT (n)	TC (n)	CC (n)	P-value
4-5	Male	126.57±27.20 (14)	130.80±25.46 (54)	129.75±19.92 (4)	P>0.05
	Female	118.13±18.35 (8)	115.75±19.40 (44)	119.17±17.75 (6)	P>0.05
	Unsexed	123.50±24.24 (22)	124.04±24.03 (98)	123.40±18.36 (10)	P>0.05
5-6	Male	135.36±32.31 (14)	144.81±32.74 (54)	145.00±34.88 (4)	P>0.05
	Female	121.88±26.72 (8)	107.39±19.45 (44)	115.00±32.56 (6)	P>0.05
	Unsexed	130.45±30.47 (22)	128.01±33.22 (98)	127.00±35.13 (10)	P>0.05
6-7	Male	145.36±31.59 (14)	146.67±28.20 (54)	138.75±28.69 (4)	P>0.05
	Female	122.50±10.35 (8)	111.70±23.72 (44)	120.83±24.38 (6)	P>0.05
	Unsexed	137.05±27.93 (22)	130.97±31.46 (98)	128.00±26.27 (10)	P>0.05
7-8	Male	137.50±32.03 (14)	134.81±39.63 (54)	141.25±24.96 (4)	P>0.05
	Female	121.25±24.16 (8)	112.05±33.78 (44)	119.17±33.83 (6)	P>0.05
	Unsexed	131.59±29.90 (22)	124.59±38.65 (98)	128.00±31.20 (10)	P>0.05
8-9	Male	182.50±42.32 (14)	181.94±44.88 (54)	187.50±18.48 (4)	P>0.05
	Female	150.63±26.65 (8)	136.25±33.41 (44)	135.00±30.50 (6)	P>0.05
	Unsexed	170.91±39.90 (22)	161.43±46.02 (98)	156.00±36.95 (10)	P>0.05
9-10	Male	145.71±24.80 (14)	134.63±32.06 (54)	132.50±9.57 (4)	P>0.05
	Female	122.50±15.81^{a} (8)	120.57±22.83^{a} (44)	88.33±37.51^{b} (6)	0.010
	Unsexed	137.27±24.38^{a} (22)	128.32±29.02^{a} (98)	106.00±36.50^{b} (10)	0.020

n: Number of samples; different superscripts within the same row indicate significant differences (P<0.05).

TGF-β2 gene polymorphism may influence the regulation of feed intake during the late growth phase.

This finding is consistent with the report of Sahib *et al.* (2021) on Malaysian native chickens, where individuals with the TT genotype of the TGF-β2 gene had higher body weights compared to the CC genotype at 12 and 13 weeks of age. This suggests that the TT genotype may be associated with higher metabolic activity. The TGF-β2 gene is known to play an important role in regulating metabolism, cell differentiation, and immune response (Li *et al.*, 2003; Muhsinin *et al.*, 2017). Higher TGF-β2 activity can enhance metabolic energy efficiency through the regulation of protein synthesis and muscle cell proliferation. Therefore, chickens with the TT genotype, which exhibit higher feed intake at the late growth stage, are presumed to have greater metabolic activity to support tissue growth.

Association of the TGF-β2 Gene with Body Weight Gain

The association analysis between the TGF-β2 gene and body weight gain (BWG) was conducted to determine the relationship between the gene's genotypes and the weekly body weight gain of Kedu chickens, as presented in Table 4.

The analysis results (Table 4) show that there were no significant differences in BWG among TGF-β2 genotypes in Kedu chickens at most observation ages. However, at 10 weeks of age, a significant difference ($P<0.05$) was observed among the TT, TC, and CC genotypes, particularly in female chickens. During this period, females with the CC genotype (86.25 g/bird/week) had significantly lower BWG compared to those with TT (115.83 g/bird/week) and TC (121.60 g/bird/week) genotypes. A similar pattern was also observed in the unsexed group, where chickens with the CC genotype (106.43 g/bird/week) showed lower BWG than those with TT and TC genotypes.

The differences in BWG among TGF-β2 genotypes are presumed to be related to the gene's role in regulating metabolism and muscle tissue growth. The TGF-β2 gene functions as an important regulator of cell proliferation and differentiation, including skeletal muscle cells (Li *et al.*, 2003; Niarami *et al.*, 2014). High TGF-β2 activity is known to enhance nutrient utilization efficiency through stimulation of protein synthesis and muscle cell growth (Li *et al.*, 2003). Therefore, chickens with TT and TC genotypes, which exhibited higher BWG, are likely to have more optimal TGF-β2 expression activity compared to those with the CC genotype.

These findings are consistent with those of Sahib *et al.* (2021), who reported that Iraqi native chickens with the TT genotype had higher BWG than those with CC at 11–13 weeks of age. Similarly, Tang *et al.* (2010) observed that Malaysian native chickens carrying the TT genotype of the TGF-β2 gene had higher body weights compared to the CC genotype at 11 weeks of age. This pattern suggests that the T allele may play a positive role in chicken growth by enhancing metabolic activity and feed utilization efficiency.

Association of TGF-β2 Gene with Feed Conversion Ratio

The feed conversion ratio (FCR) indicates the relationship between the amount of feed consumed and the resulting body weight gain. The lower the feed conversion ratio, the more efficiently the chicken converts feed into meat. The feed conversion ratio of Kedu chickens is presented in Table 5.

The analysis results (Table 5) showed that, in general, there were no significant differences in the feed conversion ratio (FCR) of Kedu chickens among the TGF-β2 genotypes at most observation ages. However, at 9–10 weeks of age, a significant difference ($P<0.05$) was found in female chickens, where the CC genotype (5.01) had a higher FCR value than the TC (3.82) and TT (4.26) genotypes. A higher FCR value indicates lower feed efficiency, suggesting that female chickens with the CC genotype tend to be less efficient in converting feed into body weight gain during the late growth phase.

The differences among TGF-β2 genotypes at 9–10 weeks of age observed in both male and female chickens indicate that genetic variation in this gene may influence feed utilization efficiency, particularly during the late growth phase. The TGF-β2 gene plays an important role in regulating cell growth, metabolism, and muscle tissue differentiation (Li *et al.*, 2003; Niarami *et al.*, 2014). Higher gene activity can enhance nutrient utilization efficiency by stimulating muscle pro-

Table 5. Association of the TGF-β2 Gene with the Feed Conversion Ratio of Kedu Chickens Aged 4–10 Weeks

Age (weeks)	Sex	TT (n)	TC (n)	CC (n)	P-value
4-5	Male	1.73±0.06 (14)	1.72±0.08 (54)	1.69±0.07 (4)	P>0.05
	Female	1.71±0.07 (8)	1.73±0.10 (44)	1.68±0.10 (6)	P>0.05
	Unsexed	1.72±0.06 (22)	1.72±0.09 (98)	1.69±0.08 (10)	P>0.05
5-6	Male	2.35±0.12 (14)	2.32±0.12 (54)	2.40±0.09 (4)	P>0.05
	Female	2.35±0.11 (8)	2.33±0.12 (44)	2.28±0.05 (6)	P>0.05
	Unsexed	2.35±0.11 (22)	2.32±0.12 (98)	2.33±0.09 (10)	P>0.05
6-7	Male	2.43±0.13 (14)	2.46±0.22 (54)	2.38±0.15 (4)	P>0.05
	Female	2.64±0.11 (8)	2.55±0.23 (44)	2.65±0.16 (6)	P>0.05
	Unsexed	2.51±0.16 (22)	2.50±0.23 (98)	2.54±0.21 (10)	P>0.05
7-8	Male	2.26±0.22 (14)	2.34±0.21 (54)	2.29±0.12 (4)	P>0.05
	Female	2.37±0.17 (8)	2.48±0.45 (44)	2.34±0.26 (6)	P>0.05
	Unsexed	2.30±0.21 (22)	2.40±0.34 (98)	2.32±0.21 (10)	P>0.05
8-9	Male	2.48±0.20 (14)	2.50±0.26 (54)	2.64±0.18 (4)	P>0.05
	Female	2.66±0.32 (8)	2.60±0.26 (44)	2.59±0.29 (6)	P>0.05
	Unsexed	2.54±0.26 (22)	2.54±0.27 (98)	2.61±0.24 (10)	P>0.05
9-10	Male	3.79±0.55 (14)	4.10±0.86 (54)	4.03±0.30 (4)	P>0.05
	Female	$4.42±0.80^{b}$ (8)	$3.88±0.52^{b}$ (44)	$5.12±1.32^{a}$ (6)	0.000
	Unsexed	$4.02±0.70^{b}$ (22)	$4.00±0.73^{b}$ (98)	$4.69±1.15^{a}$ (10)	0.027

n: Number of samples; different superscripts within the same row indicate significant differences (P<0.05).

tein synthesis and inhibiting protein degradation (Li *et al.*, 2003). Therefore, chickens with the TC genotype, which exhibited the lowest FCR value at weeks 10, are presumed to have more optimal TGF-β2 gene expression activity, allowing them to convert feed into body weight more efficiently than those with the CC genotype.

These findings support the report of Sahib *et al.* (2021), which stated that the polymorphism of the TGF-β2 gene in Iraqi local chickens is associated with growth performance and feed efficiency, where the TT and TC genotypes showed better production performance than the CC genotype. Therefore, the presence of the T allele in the TGF-β2 gene can be considered a favorable allele for improving feed efficiency in Kedu chickens, particularly during the late growth period.

CONCLUSION

The TGF-β2 gene in Kedu chickens is polymorphic, with three genotypes identified (TT, TC, and CC). The TT genotype exhibited better growth performance (body weight gain, feed intake, and feed conversion ratio) than the CC genotype, particularly at 10 weeks of age in both female and unsexed chickens.

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