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Association of *TGF-β2* Gene Polymorphism with Growth Rate in Local Chickens

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A B S T R A C T

Iraqi native chickens have tasty meat and eggs; however, they are characterized by low production efficiency. In fact, phenotypic traits, such as growth rate, are influenced by genes and environmental factors. During health and disease, a variety of cellular processes such as proliferation, differentiation, motility, adhesion, migration, apoptosis, and immune response regulate the *TGF-β* genes. The enhancement in body weight can be reached through mass selection, whereas feed conversion ratio (FCR) is relatively more difficult to improve. This means, selecting for body weight has been submitted as an effective way of indirectly improving feed conversion ratio. Therefore, the present study attempts to identify associations between productive traits and polymorphism of *TGF-β2* gene in local Iraqi chicken. Seventy-five male birds were used in this study. The restriction enzyme RsaI has been used to detect the target region (284 bp) in the *TGF-β2* gene. A single nucleotide polymorphism (SNP) was identified at the position 62 in the exon 1 region of *TGF-β2* by using PCR-RFLP and DNA sequencing technique. The genotypic frequencies were 46.7, 40, and 13.3% for CC and TC and TT genotypes, respectively. While the allele frequency of C and T were 0.67 and 0.33%, respectively. Generally, during the last period of rearing the best significant (P<0.05) improve in the body weight, weight gain and FCR were recorded in the TT genotype of the *TGF-β2* gene. In conclusion, a functional sequence in the genome could be attributed to the mutation. Therefore, genotype of the *TGF-β2* gene could be exploited to select the best individual as a parent to the next generations for improving of growth rate in local chickens.

Keywords: chicken, *TGF-β2* gene, single nucleotide polymorphism, productive performance

INTRODUCTION

The native domestic breeds of chicken are in danger of extinction in Iraq, as they are in many other parts of the world, so restoration and protection of these potentially important genetic resources would take a lot of focus and effort (1, 2). Native chickens, on the other hand, because of their tasty eggs and meat, as well as their ability to survive a wide range of infectious diseases and high temperatures, play an important role in the socio-economic aspects of smallholder societies (3). One of the most significant challenges in native chicken production is growth. Native chickens in Iraq achieve slaughter weights of 4.5 months or more due to an intensive rearing regime

(4). This is in stark contrast to broiler chickens (Ross 308 strains), which can weigh up to 1.4 kg in just 28 days (5, 6). The final expression of growth is the product of interactions among genetic, nutritional, and environmental factors (7). The genetic regulation of growth is complex and learning the molecular processes of growth will contribute to more efficient selection for growth in chickens (8). The use of molecular biotechnology in conjunction with an effective selection program would enhance native and local livestock production, adaptability to the climate, and preserve genetic diversity (9). The most efficient approach at improving livestock processing productivity is to use animals that are genetically superior in terms of cumulative genes. Selection based on the genome of animals requires a DNA test that is costly and time-consuming. As a result, increasing our understanding of gene polymorphisms and their relationship to essential economic traits contributed to the discovery of effective alleles and their use as molecular markers in selection programs. Therefore, more research is required before using a single nucleotide polymorphism (SNP) for marker assisted selection, such as expanding the size of the sample population, using multiple species simultaneously and the same maintenance (10). The transforming growth factor subfamily is one of the most important groups of genes involved in the development of growth and fitness traits (11, 12). The chicken transforming growth factor-beta 2 (TGF-β2) is found on chromosome 3 (13, 14). The transforming growth factor-beta 2 is a family of growth factor hormones that regulate the biological activity across a wide range of processes, including morphogenesis (15), development (16), production (17), reproduction (18), and disease resistance (18). Marker assisted selection is a new breeding technique that uses molecular markers to save time and resources by speeding up the breeding process (19). As a consequence, it is important to find and develop new markers that could be used in breeding. Therefore, this research, which aimed to understand the genetic diversity at the genomic level (DNA) rather than at the phenotypic level (measuring traits in birds), would serve as the foundation for selective breeding to help Iraqi chicken production develop.

MATERIALS AND METHODS

Experimental Birds

This study was carried out at a poultry farm, College of Veterinary Medicine, University of Baghdad during the period of 9 weeks from 7th March - 6th May 2019. A total of 75 male chicks (Iraqi local) at age 28 days old were brought from the Poultry Research Station, Ministry of Agriculture located at Abu Ghraib, Baghdad, Iraq. All birds were numbered by metal figures fixed on the wing pad and randomly divided into plots. Diet was provided ad libitum (Table 1). The birds were cared for in accordance with animal welfare principles and with ethics committee approval.

Table 1. Composition of grower diet according to (20)

	Diet (%)	
Ingredients	**4-10 weeks**	**11-13 weeks**
Yellow corn	29.6	23.2
Wheat	40	36
Soybean meal (45% CP?)	13.5	11
Barley	10	23
Protein concentrate for broiler	5	0
Protein concentrate for layer	0	5
Dicalcium phosphate	0.8	0.4
Limestone	0.8	1.1
Salt	0.3	0.3
Total	100	100
Calculated nutritional content		
Metabolized energy (kcal/kg)	2938.4	2881.35
Crude protein (%)	16.7	15.92
Methionine + Cysteine (%)	0.70	0.67
Lysine (%)	0.80	0.78
Fat (%)	2.7	2.56
Fiber (%)	3	3.39
Calcium (%)	0.80	0.82
Available phosphorus	0.50	0.33

Sample Collection and Laboratory Analysis

One ml of blood was collected at age 90 days old from the brachial vein of all experimental birds by using disposable syringes. The samples were put in EDTA tubes kept in freezer (-20 °C) for the molecular tests.

A DNA extraction kit was used to obtain DNA from the blood (Favorgen, Taiwan). The primers were supplied from Alpha DNA Canada, as forward 5'- GCC ATA GGT TCA GTG CAA G-3' and reverse 5'- TGA CAG AAG CTC TCA AGC C-3' (21-23). The components of PCR reaction were prepared according to the procedure suggested by the manufacturing company (Promega, USA) using 12.5 µL master mix, 1 µL forward primer, 1 µL reverse primer, 3 µL of DNA and 7.5 µL distilled water. The optimum conditions for the gene amplification included initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, annealing at 54 °C for 30 sec, extension at 72 °C for 30 sec, and then final extension at 72 °C for 7 min. The PCR-RFLP assay included digesting the of 20 µL PCR products with 1 µL of RsaI restriction enzyme at 37 °C for 3 h. The restriction patterns were visualized under ultraviolet illumination on a 1.5% agarose gel electrophoresis stained with ethidium bromide. In addition, the 20 µL of PCR products were sent to Macrogen company, Korea, for sequencing.

Production Traits

The electronic balance (Guangdong, China) was used to measure live body weight and feed consumption to calculate body weight gain and feed conversion ratio at weekly intervals (24).

Statistical Analysis

The statistical analysis system (SAS) program was used to investigate the effect of genotype of the *TGF-β3* gene on the body weight (25). To compare means, the general linear model procedure and Duncan's multiple range test were used. In addition, the distribution ratios of the herd and the frequency of the alleles were obtained by chi-square test based on Hardy-Weinberg law (26).

RESULTS AND DISCUSSION

DNA Extraction

The DNA extracted was very efficient and showed sharp bands on the agarose gel (Figure 1).

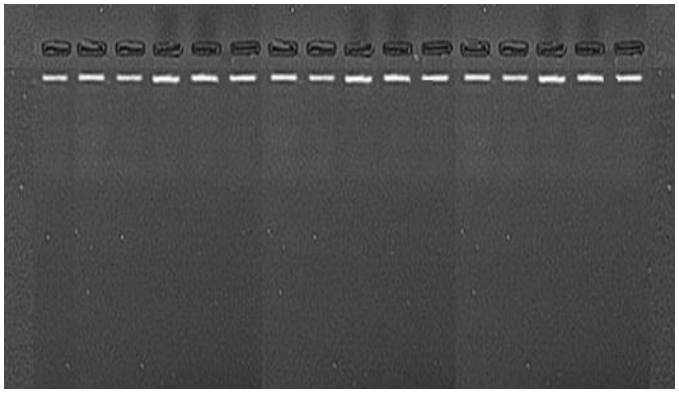

Figure 1. Genomic DNA in 1.5% agarose gel

PCR Assay

Polymerase chain reaction (PCR) amplified a partial region of the TGF-β2 gene, which showed a molecular weight of 284 bp (Figure 2). The present results agree with other authors (21-23).

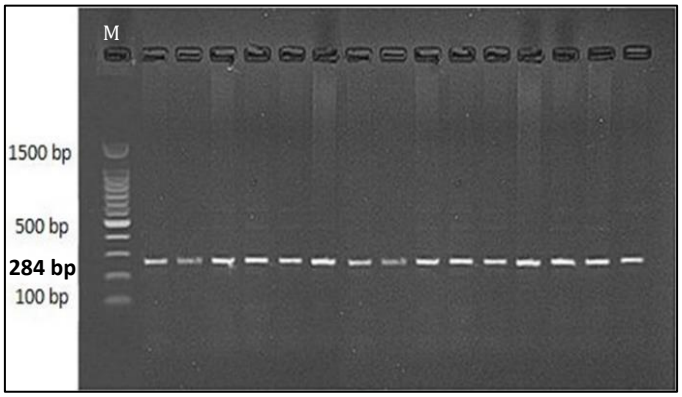

Figure 2. Electrophoresis of the PCR amplified *TGF-β2* gene shows bands of approximately 284 bp in 1.5% agarose gel. M= 100 bp ladder

PCR-RFLP Assay

The PCR products were subjected to restriction digestion using the RsaI enzyme (GT/AC) to detect SNP T>C in the *TGF-β2* gene's first exon and it was able to cut at this position only when the SNP was present (when T was transformed to C). The following fragment sizing patterns were observed by agarose gel electrophoresis (Figure 3)

1. Wild type CC: No cleavage of the whole 284 bp segment by RsaI.

2. Heterozygous TC: RsaI cut the sequence to show three fragments in agarose gel electrophoresis (284 bp, 184 bp and 100 bp).

3. Homozygous TT: RsaI cut the sequence to show two fragments in agarose gel electrophoresis (184 bp and 100 bp)

The current result agrees with other researchers (21-23).

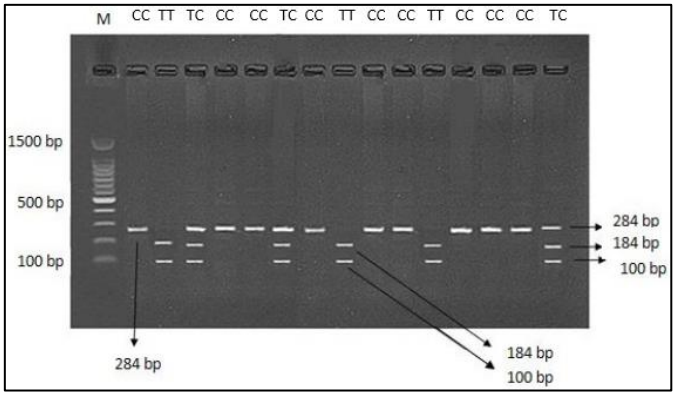

Figure 3. RsaI restriction fragment patterns of TGF-β2 by PCR-RFLP on 1.5% agarose gel. M= 100 bp ladder; TT, TC, CC = genotype

Sequencing

The genotypes CC, TC and TT of the TGF-β2 gene were observed in local chicken. At the same time, in exon 1, a T/C transition mutation was discovered at location 62 bp (Figure 4).

The substitution of thymine (T) by cytosine (C) in chickens resulted in a change in the RsaI restriction site sequences, which were changed from GT|AC to GC|AC, preventing the RsaI enzyme from recognizing its restriction site (21-23).

Distribution of Genotype and Allele Frequency

Table 2 shows different genotypes of the *TGF-β2* gene encoding region due to different genetic bundles resulting from enzymatic digestion. The genotypes were CC, TC and TT, and their distribution ratios were 46.7, 40 and 13.3%, respectively. Moreover, the differences between these percentages were highly significant ($P<0.01$). According to Hardy-Weinberg law, allele frequencies were 0.67 and 0.33 for both C and T, respectively. The chi-square analysis revealed that the association of RsaI allelic pattern with strain is significant. This difference can be due to factors, such as cross breeding between populations, gene flow across boundaries and different sample numbers used.

Cross breeding leads to increased heterozygosity among

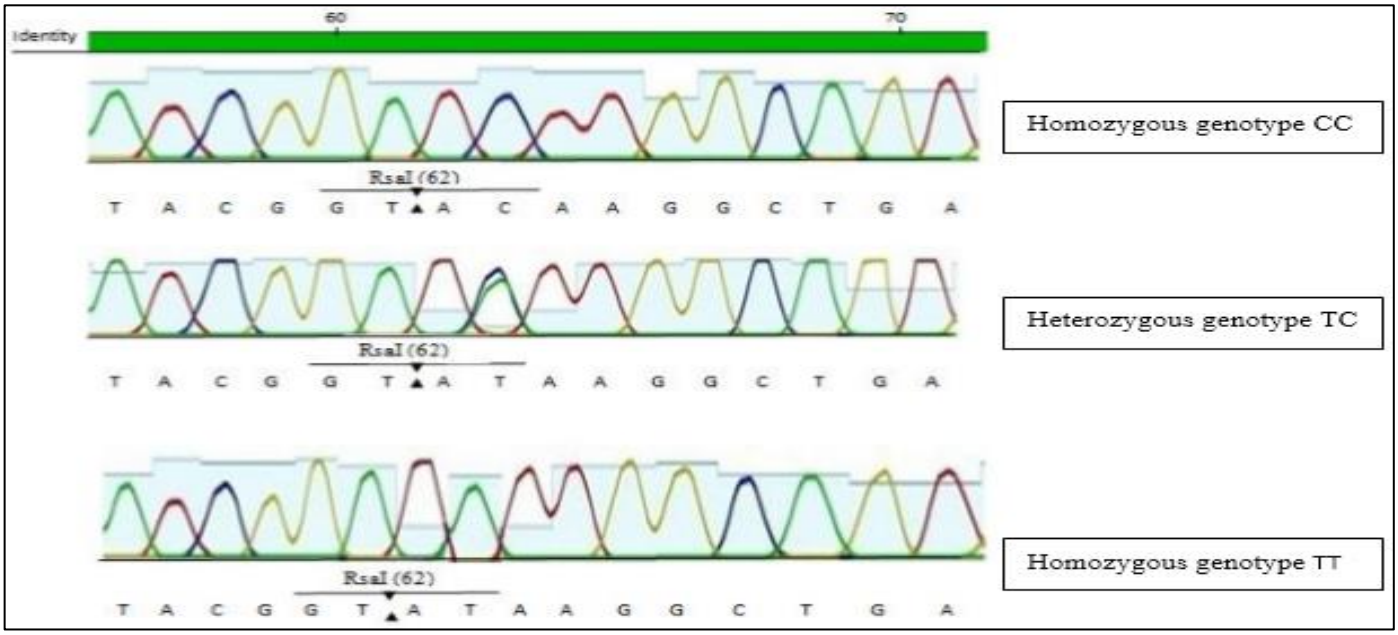

Figure 4. Sequence analysis of the *TGF-β2* gene

populations and sub-populations (27). Gene flow across boundaries introduces new unique alleles in a population, leading to an increase in heterozygosity for the whole population (27). It was reported that chickens had two alleles (T and C) and three genotype variants (TT, CT, and CC) for the *TGF-β2* gene (21). Polymorphic Indonesian chickens with two alleles (T and C) and three genotypes (TT, CT, and CC) were seen by (23). At the same time, the T allele had a higher prevalence than the C allele in Indonesian chickens owing to inbreeding and vigorous selection of the *TGF-β2* gene.

Table 2. Distribution of genotype and allele frequency *TGF-β2* gene

Genotype	Number	Percentage
CC (Wild)	35	46.7
TC (Heterozygous)	30	40
TT (Mutant)	10	13.3
Total	75	
χ^2	11.320**	

Allele	Frequency
C	0.67
T	0.33

**P<0.01

Effect of the *TGF-β2* Gene on the Body Weight

The results of Table 3 show no significant differences among the three genotypes of the *TGF-β2* gene in weekly body weight of males in the fourth, fifth, sixth, seventh, eighth, ninth and tenth week. While there were significant differences (P<0.05) in body weight in week eleven, twelve and thirteen by TT genotype that was dominant on the other genotypes 1003, 1191, and 1304 g, followed by TC genotype 954, 1139, and 1246 g, then CC genotype 918, 1077, and 1176 g, respectively. These values reflect how diverse the indigenous chickens in Iraq are within their ecotypes. The increment of body weight may be related to genotype.

In reality, the number and size of an organ's or tissue's cells and extracellular components determine its size. The number and size of muscular cells are determined by a number of factors, both directly and indirectly. Steroids, TGF-β, and myostatin are the three primary hormones that regulate muscle development in livestock (28).

The transforming growth factor-beta is part of a broad family of multifunctional growth factors that play a pivotal role in all tissues of the body for embryo and adult (11, 29, 30). The profile of polypeptides from the *TGF-β* family includes stimulation or inhibition of proliferation, differentiation, motility, adhesion, migration, apoptosis and immune response of epithelial, endothelial, hematopoietic, neural and mesenchymal cells (31-33). These functions participate in the control of normal tissue homeostasis or pathological conditions (34-36). Therefore, the *TGF-β* genes influence many different cell types, such as myogenesis, chondrogenesis, osteogenesis, hematopoiesis, epithelial cell differentiation, and adipogenesis (37-41).

At stage 10 days of embryo development, chicken *TGF-β2* mRNA was found in cells from all three germ layers (42, 43), implying that it plays a significant role in the development of many tissues. The *TGF-β* genes are expressed in a variety of locations in the early avian embryo, and they may play a role in phenotypic transformation, matrix deposition, and cell proliferation (44). The TGF-β genes can act as a stimulator for satellite cell proliferation and differentiation, resulting in myofibrillar hypertrophy and muscle tissue regeneration (45). Expression of *TGF-β* is decreased in the transitional chondrocytes of chicks with tibial dyschondroplasia, but TGF-β expression is increased where the lesion is being healed (46).

Table 3. Relationship of genotypes of the *TGF-β2* gene with body weight (g). Mean ± standard error

	Genotype		
Body weight at	**CC (No. =35)**	**TC (No. =30)**	**TT (No. =10)**
Week4	362 ± 8.37 [a]	366 ± 7.46 [a]	361 ± 17.1 [a]
Week5	377 ± 8.49 [a]	382 ± 7.64 [a]	377 ± 18.4 [a]
Week6	399 ± 8.93 [a]	403 ± 7.85 [a]	401 ± 18.5 [a]
Week7	444 ± 9.83 [a]	447 ± 8.08 [a]	444 ± 18.2 [a]
Week8	517 ± 10.6 [a]	520 ± 8.51 [a]	519 ± 18.4 [a]
Week9	624 ± 11.1 [a]	628 ± 8.66 [a]	625 ± 19.3 [a]
Week10	758 ± 11.7 [a]	761 ± 8.88 [a]	758 ± 20.1 [a]
Week11	918 ± 9.88 [c]	954 ± 5.35 [b]	1003 ± 12.6 [a]
Week12	1077 ± 7.77 [c]	1139 ± 5.44 [b]	1191 ± 12.4 [a]
Week13	1176 ± 8.40 [c]	1246 ± 5.60 [b]	1304 ± 12.8 [a]

Significant variations at P<0.05 are denoted by small separate letters in the same row

The *TGF-β2* mRNA was identified in cultured chicken embryo cardiac myocytes as well as in developing chicken embryo heart and muscles (43, 47). Also, *TGF-β2* is prevalent in the tissues that make up the skeleton, where it aids in bone growth regulation, and in the intricate lattice that exists in the spaces between cells (the extracellular matrix) (48-50).

Du et al. (2013) (11) observed that TGF-β2 polymorphism was related to skeletal traits. (51) revealed a significant association of TGF-β2 with body weight and explained its role in bone resorption and remodeling. The study of (16) showed that TGF-β2 may play an important role in fetal myoblast proliferation in chicken leg muscles.

The results of this study agreed with other authors (30, 52, 53).

Effect of the *TGF-β2 gene* on Weight Gain

Table 4 shows non-significant differences among the three genotypes of the *TGF-β2* gene in weekly weight gain of males in the fifth, sixth, seventh, eighth, ninth and tenth week. While, there were significant differences in weight gain in the eleven, twelve, thirteen week and total by TT genotype that was dominant on the other genotypes 244.50, 188, 113 and 942.50 g, followed by TC genotype 193.26, 184.84, 107.56 and 880.76 g, then CC genotype 160.03, 158.48 99.94 and 814.88 g, respectively.

The improvement occurred in total weight gains may be related to genotype. The significant differences in body weight between the various genotypes of the *TGF-β2* gene led to the presence of differences between the various genotypes in the trait of body weight gain. The current results agree with (30).

Effect of the *TGF-β2* gene on Feed Intake

Data obtained from this study are presented in Table 5. Genotypes of the *TGF-β2* gene had no significant difference in feed intake at the sixth, tenth week and total. But there were significant differences in feed intake in the fifth, seventh, eighth, ninth, eleven, twelve and thirteen week.

There was no significant difference observed in the total feed intake, which may support the hypothesis that local chickens exhibit decreased feed intake, meaning that heritability value for feed intake in chicken is low (54).

Table 4. Relationship of genotypes of the *TGF-β2* gene with weight gain (g). Mean ± standard error

	Genotype		
Weight gain at	**CC (No. =35)**	**TC (No. =30)**	**TT (No. =10)**
Week5	15 ± 0.43 [a]	16 ± 0.63 [a]	16 ± 1.66 [a]
Week6	22 ± 0.75 [a]	22 ± 0.77 [a]	23 ± 1.49 [a]
Week7	45 ± 1.18 [a]	44 ± 0.92 [a]	43 ± 1.04 [a]
Week8	73 ± 0.86 [a]	73 ± 0.73 [a]	75 ± 0.89 [a]
Week9	107 ± 0.67 [a]	108 ± 0.89 [a]	106 ± 1.62 [a]
Week10	134 ± 0.91 [a]	133 ± 0.94 [a]	133 ± 1.52 [a]
Week11	160 ± 4.52 [c]	193 ± 9.30 [b]	245 ± 21.8 [a]
Week12	159 ± 4.05 [b]	185 ± 0.86 [a]	188 ± 1.29 [a]
Week13	100 ± 1.23 [c]	108 ± 1.03 [b]	113 ± 1.13 [a]
Total	815 ± 3.83 [b]	881 ± 1.76 [ab]	943 ± 47.9 [a]

Significant variations at P<0.05 are denoted by small separate letters in the same row

Effect of the *TGF-β2* gene on feed conversion ratio

Generally, Table 6 shows non-significant differences among the three genotypes of the *TGF-β2* gene in weekly feed conversion ratio (FCR) in the fifth, sixth, seventh, eighth, and tenth week. While there were significant differences in FCR in the ninth, eleven, twelve, thirteen week and final.

Feed conversion ratio is one of the economic important parameters in poultry production because it includes feed consumption and weight gain (17, 55, 56). Therefore, any

improvement of FCR may be resulted from improvement in body weight gain during the period of study (Table 4).

The results obtained in this study support the broad effects of the *TGF-β2* gene on growth and development. At the same time, gene polymorphism may be used as a candidate of quantitative trait loci to improve productivity in local chickens.

Table 5. Relationship of genotypes of the *TGF-β2* gene with feed intake (g). Mean ± standard error

	Genotype		
Feed Intake	**CC (No. =35)**	**TC (No. =30)**	**TT (No. =10)**
Week5	62 ± 1.60 [ab]	57 ± 1.10 [a]	64 ± 0.35 [b]
Week6	103 ± 2.45 [a]	102 ± 2.60 [a]	97 ± 2.35 [a]
Week7	236 ± 2.50 [b]	232 ± 1.45 [ab]	224 ± 1.60 [a]
Week8	349 ± 1.00 [b]	334 ± 3.45 [a]	340 ± 0.55 [b]
Week9	435 ± 1.75 [a]	454 ± 2.90 [b]	447 ± 1.95 [a]
Week10	562 ± 2.05 [a]	551 ± 4.90 [a]	569 ± 4.90 [a]
Week11	683 ± 2.00 [c]	673 ± 2.55 [b]	657 ± 1.50 [a]
Week12	784 ± 3.90 [a]	766 ± 3.05 [a]	793 ± 4.50 [b]
Week13	874 ± 2.25 [a]	894 ± 3.85 [a]	905 ± 4.50 [b]
Total	4087 ± 8.50 [a]	4063 ± 14.9 [a]	4094 ± 7.40 [a]

Significant variations at P<0.05 are denoted by small separate letters in the same row

Table 6. Relationship of genotypes of the *TGF-β2* gene with feed conversion ratio (FCR, g:g). Mean ± standard error

	Genotype		
FCR at	**CC (No. =35)**	**TC (No. =30)**	**TT (No. =10)**
Week5	4.103 ± 0.018 [a]	3.577 ± 0.091 [a]	3.904 ± 0.489 [a]
Week6	4.602 ± 0.286 [a]	4.682 ± 0.109 [a]	4.160 ± 0.278 [a]
Week7	5.222 ± 0.118 [a]	5.258 ± 0.092 [a]	5.200 ± 0.013 [a]
Week8	4.778 ± 0.038 [a]	4.584 ± 0.186 [a]	4.515 ± 0.043 [a]
Week9	4.072 ± 0.007 [a]	4.215 ± 0.001 [b]	4.213 ± 0.017 [a]
Week10	4.191 ± 0.055 [a]	4.153 ± 0.012 [a]	4.278 ± 0.001 [a]
Week11	4.267 ± 0.034 [b]	3.482 ± 0.003 [ab]	2.685 ± 0.521 [a]
Week12	4.947 ± 0.118 [b]	4.144 ± 0.011 [a]	4.215 ± 0.028 [b]
Week13	8.742 ± 0.122 [b]	8.313 ± 0.074 [b]	8.004 ± 0.025 [a]
Final	5.015 ± 0.034 [b]	4.613 ± 0.026 [ab]	4.343 ± 0.213 [a]

Significant variations at P<0.05 are denoted by small separate letters in the same row

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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علاقة تعدد المظاهر الوراثية لجين عامل النمو المحول بيتا 2 مع معدل النمو في الدجاج المحلي

علي مهدي صاحب[1]، عباس فوزي الخالصي[2]، و مشتاق طالب عبد الواحد[2]

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الخلاصة

يتميز الدجاج المحلي في العراق باللحم و البيض ذات الطعم المرغوب. الا انه، ذو كفاءة انتاجية منخفضة. في الواقع، تتأثر الصفات المظهرية مثل معدل نمو الجسم بالعوامل الوراثية (الجينات) والعوامل البيئية. تنظم جينات عامل النمو المحول بيتا عددًا من العمليات الخلوية مثل الانتشار والتمايز والحركة والالتصاق والهجرة والاستماتة والاستجابة المناعية أثناء الحالة الصحية والمرضية. ان الزيادة في وزن الجسم يمكن الحصول عليها من خلال الانتخاب المستمر ، في حين يصعب تحسين كفاءة تحويل الغذائي. هذا يعني أن الانتخاب لوزن الجسم يعد كطريقة غير مباشر فعالة لتحسين كفاءة التحويل الغذائي. لذا أجريت الدراسة الحالية لمعرفة العلاقة بين تعدد المظاهر الوراثيه لجين عامل النمو المحول نوع بيتا 2 و الصفات الانتاجية. اذ طبقت الدراسة على 75 طير ذكر. حيث استخدام إنزيم القطع RsaI للكشف عن المنطقة المستهدفة (284 قاعدة نايتروجينية) في جين عامل النمو المحول بيتا 2. تم تحديد تعدد أشكال النوكليوتيدات المفرد في الموقع 62 لمنطقة الاكسون الاول لجين عامل النمو المحول بيتا 2 باستخدام تقنية تعدد مظاهر اطوال القطع المقيدة و تتابع تسلسل الحمض النووي. أظهرت النتيجة على التوالي ان توزيع التراكيب الوراثية كانت 46.7، 40 و 13.3% للمظاهر الوراثيه CC، TC و TT. كما لوحظ ان توزيع التكرار الاليلي كان 0.67 و 0.33 % على التوالي للاليل C و T. بصورة عامة، تبين في نهاية فترة الدراسة ان هناك تأثير معنوي من جين عامل النمو المحول بيتا 2 على وزن الجسم،الزيادة الوزنية و كفاءة التحويل الغذائي فقد سجل المظهر الوراثي TT اعلى المعدلات. لذا يستنتج، ان الطفرة ممكن ان تغير وظيفة الجين لذالك يمكن استغلال جين عامل النمو المحول بيتا 2 في برنامج انتخاب افضل الافراد عبر عدة اجيال لتحسين النمو لدى الدجاج المحلي.

الكلمات المفتاحية: الدجاج، جين عامل النمو المحول نوع بيتا 2،تعدد أشكال النوكليوتيدات المفرد، الاداء الانتاجي