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Quantitative Trait Loci for Body Weight Associated with Transforming Growth Factor-beta 3 Gene in Local Chickens

[1]Ali Mahdi Sahib , [2]Abbas Fawzy Al-Khalisy
[1]Department of Public Health, College of Veterinary Medicine, University of Kufa, Iraq
[2]Department of Public Health, College of Veterinary Medicine, University of Baghdad, Iraq
E-Mail: alim.alkaabi@uokufa.edu.iq

Abstract
Local chickens in Iraq are characterized by low production efficiency. In fact, phenotypic traits such as growth rate is influenced by genes and environmental factors. However, the present study attempts to identify associations between growth rate and polymorphism of TGF-β3 gene in Iraqi local chicken. Seventy-five male birds were used in this study. The restriction enzyme BsrI has been used to detect the target region (1078 bp) in the TGF-β3 gene. A SNP was identified at 9361 position in the exons 3-6 region of TGF-β3 by using a DNA sequencing technique. The genotypic frequencies were 66.67, 17.33 and 16% for AA and CA and CC genotypes respectively. While, the allele frequency of gene A and C were 0.75 and 0.25%, respectively. Generally, during the last period of rearing we noted the best improved significantly ($p>0.05$) for body weight was recorded in the CA genotype of TGF-β3 gene. In conclusion, the TGF-β3 gene may have broad effects on growth and development. At the same time, polymorphism may be used as a candidate genes of QTL to improve body weight in local chickens.

Keywords: Chicken, Transforming growth factor beta-3, Sequence technique, Body weight.

Introduction
The poultry industry is successful in providing high quality, affordable food (Abdulwahid, 2015; Batkowska *et al*., 2017; Zhang *et al*., 2018). The term "local," "indigenous," or "native" chickens refers to a community of birds that originated in a particular region and have adapted to its unique climate (Al-Khalaifa *et al*., 2013). In Iraq, as in many areas of the world, there is a risk of extinction of the native domestic breeds of chicken, so great attention and efforts are required for the conservation and preservation of these potentially valuable genetic resources (Yacoub *et al*., 2013; Yacoub *et al*., 2015). The variation of the sequence within the information of the species on genetic diversity and the relationships between populations, individuals, breeds of animals, and species is important for breeders for the improvement of animal breeds, for conservation, and for the study of population evolutionary ecology (Vigouroux *et al*., 2005). Studies on genetic diversity can identify alleles that could influence

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the body's ability to survive in its existing habitat, or could allow it to survive in multiple habitats. This knowledge is valuable for the conservation of germ plasm, the population, the individual, the variety or identification of the breed (Bennett *et al*., 2007). The evolution of a multicellular organism into ever more complex life forms needs the establishment of communication and control among individual cells to maintain order in the organism. The basic physiological processes, including proliferation, differentiation, metabolism, and apoptosis, are intricately regulated by a dense signaling network that is elicited by cytokines, growth factors or polypeptide hormones. Among those polypeptide/hormone-induced signals, the transforming growth factor-beta (TGF-β) family is particularly important (Crane and Cao, 2014). The TGF-β superfamily members are multifunctional cell-cell signaling proteins that play pivotal roles in tissue homeostasis and development (Saito *et al*., 2018). The chicken TGF-β3 maps to chromosome 5 (Abasht *et al*., 2006; Wang *et al*., 2012). Well, it consists of 7 exons, 6 introns and spans 16-kb of the chicken genome. The identified quantitative trait loci (QTL) can then be characterized at the molecular level to detect the single nucleotide polymorphism (SNP) potentially associated with production traits (Xie *et al*., 2012; Abdalhag *et al*., 2015). These SNPs can be used to design relatively easy, fast and time-saving marker-assisted selection tests. Single nucleotide polymorphisms are variations of a single base with a frequency of at least 1% and are used mainly as markers for the mapping of the whole genome (Baslasubramanian *et al*., 2005). Amirinia *et al,* (2011) indicated that a SNP marker in the TGF-ß3 gene was associated with body composition traits and a potential marker for molecular marker-assisted selection programs in commercial broilers. Chen *et al*., (2013) found polymorphism in the chicken TGF-β genes had a significant effect on myofiber characteristics. Jin *et al*., (2013) identified SNP in TGF-β3 gene is significantly associated with body weight. Recently, a new SNP has been identified in the TGF-β3 gene which have a positive effect on chicken growth (Tang *et al*., 2011; Hosnedlova *et al*., 2020). At the same time, studies and researchers in Iraq for improving the productivity of local chickens are still limited (Abdulwahid *et al*., 2019; Abdel Amir *et al*., 2019; Mohammed, 2019). As a reason, the current study's aim was to see whether there was a correlation between TGF-β3 gene polymorphism and body weight.

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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 seventy-five male chicks (Iraqi local) at 28 days old were brought from the poultry research station Abu Ghraib/Ministry of Agriculture. All birds were numbered by metal figures fixed (installed) on the wing pad and randomly divided into cages. Diet was provided *ad libitum* according to (NRC, 1994). Birds were maintained according to the principle of animal welfare and approval of an ethics committee.

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 the freezer (-20 ºC) for molecular tests. DNA was extracted from blood by using a DNA extraction kit (Favorgen, Taiwan). The primers were supplied from (Alpha DNA, Canada), as forward 5'-CGG CCT GGA AAT CAG CAT AC-3' and reverse 5'- GAA GCA GTA GTT GGT ATC CAG-3' (Malek and Lamont 2003; Tohidi *et al*., 2013). The components of PCR reaction were prepared according to the procedure that was 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 distill water. The optimum condition for gene detection includes initial denaturation at 95 ºC for 5 minutes; followed by 30 cycles of denaturation at 95 ºC for 30 seconds, annealing at 54 ºC for 30 seconds, extension at 72 ºC for 30 seconds and then final extension at 72 ºC for 7 minutes. In addition, 20 µl of PCR products were sent to a company (Macrogene, Korea) for sequencing.

Productive parameter

Live body weight was calculated at weekly intervals by using electronic balance (Guangdong, China) according to (Al-zubaidie, 1986).

Statistical analysis

The statistical analysis system program was used to investigate the effect of genotype of the TGF-β3 gene on body weight (Cary, 2012). To compare meanings, the general linear model procedure and Duncan's multiple range test were used. As well as, extracting the distribution ratios of the herd and the frequency of the alleles obtained by chi-square test of gene based on Hardy-Weinberg law (Edwards, 2008).

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Results and Discussion

DNA extraction

The DNA extracted was very efficient and showed a sharp band Figure (1).

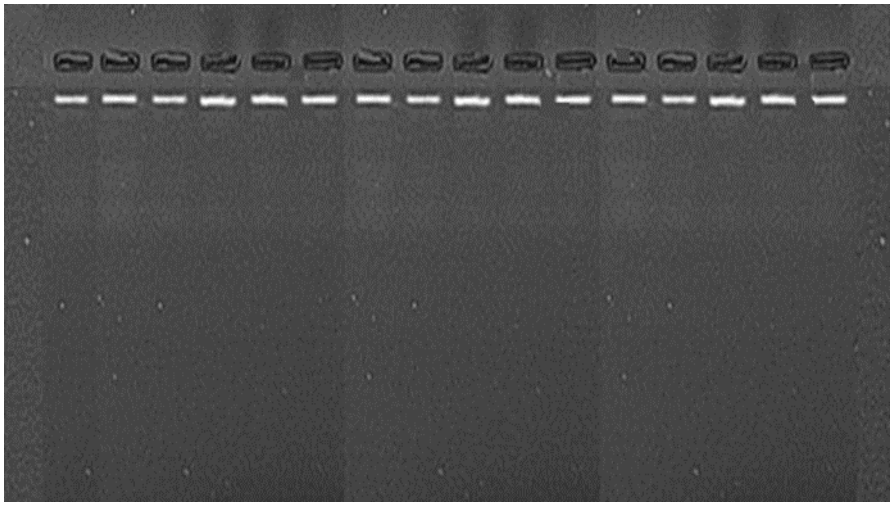

Figure (1): Genomic DNA in 1.5% agarose gel

PCR assay

Polymerase chain reaction (PCR) amplified the region of the TGF-β3 gene, which showed a molecular weight of 1078 bp. Figure (2). The present results agree with Malek and Lamont 2003; Tohidi *et al*., 2013.

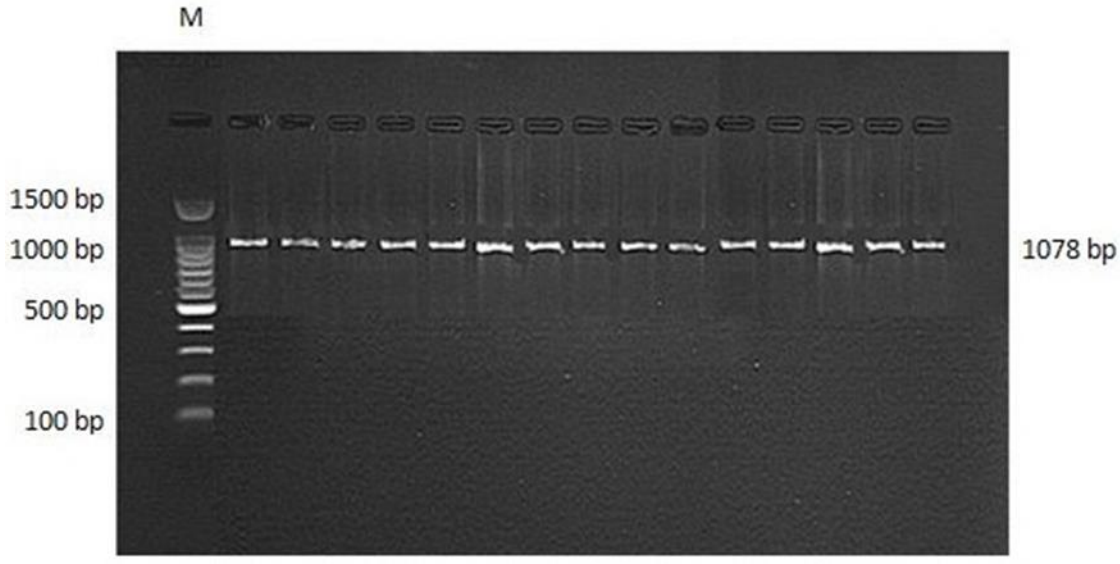

Figure (2): Amplification TGF-β3 gene in 1.5% agarose gel. M= 100 bp ladder.

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Sequence assay

The genotypes of TGF-β3 gene in local chicken were observed AA, CA and CC respectively. At the same time, C/A transition mutation was found at position 9361 bp in the exon 3-6 Figure (3).

Sequence variation in the TGF-β3 gene may be related to the change in response of the gene to their functions.

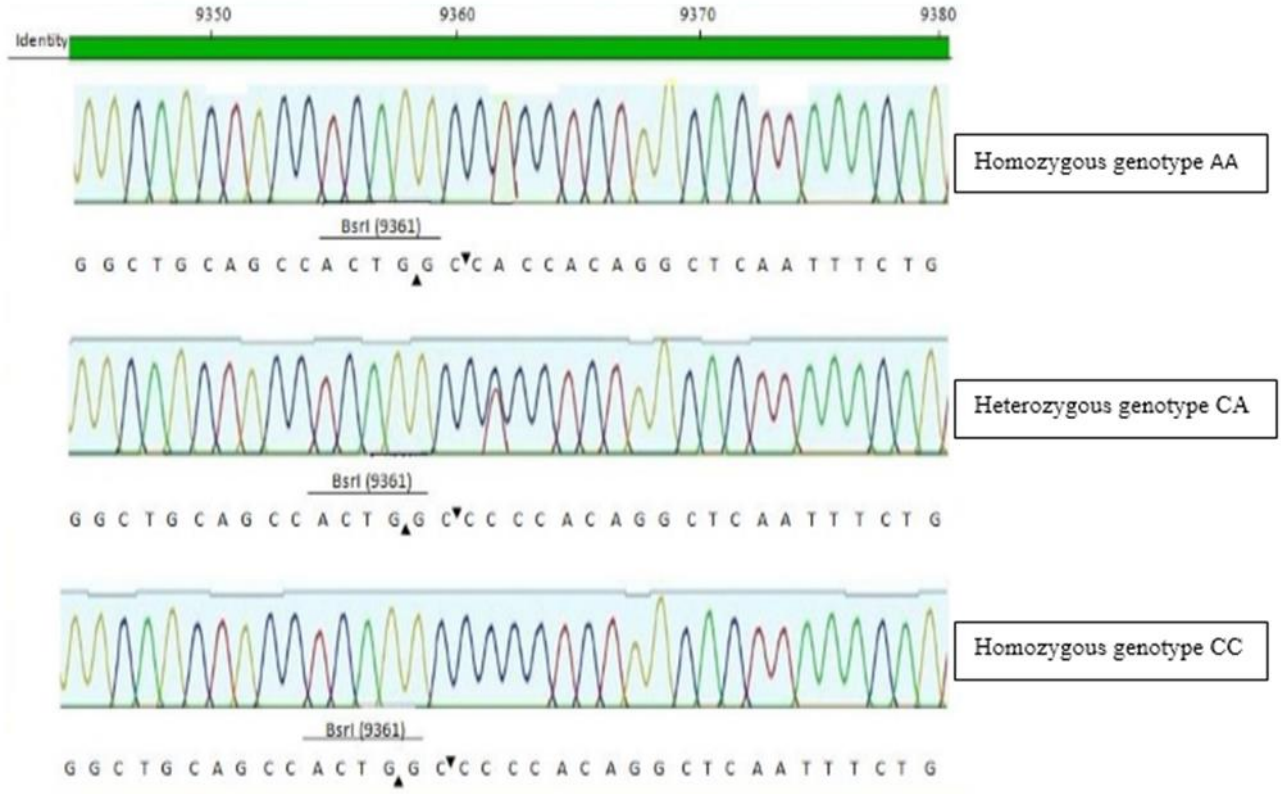

Figure (3): Sequence analysis of TGF-β3 gene

Distribution of genotype and allele frequency

The results in Table (1) refer to the distribution of different genotypes of TGF-β3 gene in local chicken that revealed significant variations ($P<0.01$) in the rate of three genotypes. The genotype AA had the highest percentage (66.67%), followed by genotype CA (17.33%), and genotype CC had the lowest percentage (16%), and the allele frequency of A was dominant on allele frequency of C that reached 0.75 and 0.25 as A and C allele respectively, As the law of Hardy-Weinberg. The chi-square analysis revealed that the association of BsrI allelic pattern with strain is significant.

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Based on the results of this study, it can be concluded that differences in the level of SNP in chickens may be due to differences in sample sizes, differences in genetic potential of species and implementation of breeding programs. Previous studies of Malek and Lamont (2003); Tohidi, *et al*., (2013); Khaerunnisa, *et al*., (2020) indicated that native chickens were polymorphic with two alleles found (A and C) and three genotypes (AA, CA, and CC).

Table (1) Distribution of genotype and allele frequency of TGF-β3 gene.

Genotype	Number	Percentage (%)
AA (Wild)	50	66.67
CA (Heterozygous)	13	17.33
CC (Mutant)	12	16
Total	75	
Chi-square (X^2)	12.934 *	
Allele	Frequency	
A	0.75	
C	0.25	
* ($p<0.01$).		

Effect of TGF-β3 gene on body weight

Table (2) shows that there are no significant differences in weekly body weight of males in the fourth, fifth, sixth, seventh, eighth, ninth, and tenth weeks between the three genotypes of the TGF-β3 gene. While, there were significant differences in body weight at eleven, twelve and thirteen week by CA genotype that was dominant on the other genotypes 998.69, 1185.30 and 1297.92 gm, followed by CC genotype 963.08, 1142.58 and 1249.75 gm then AA genotype 924.64, 1092.48 and 1194.58 gm respectively.

All These results may suggest a positive association between the TGF-β3 SNP and the high body weight of chickens. The scientific principle of this association is based on the facts which indicate that variability within genes coding for protein products involved in key physiological mechanisms and metabolic pathways is directly or indirectly involved in determining economic traits, which might probably explain a fraction of the genetic variability for the production trait itself (Fontanesi *et al*., 2008). Davies *et al*. (2002) reported that sense mutation can change the gene expression, which in turn a different protein with different characterizes is created as a result of amino acids change. This protein may lose its function or become activated or exhibit a new function. It is possible that the variation happened in amino acids due to the TGF-β mutations caused a significant change in the TGF-β function. The TGF-β3 protein is especially abundant in tissues that develop into the muscles

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used for movement (skeletal muscles), and plays a key role in their development (Reinhoff *et al*., 2013). The study of Lu *et al*. (2013) showed that TGF-β3 may play an important role in fetal myoblast proliferation in chicken leg muscles. The TGF-β proteins act as autocrine and paracrine regulators of adipocyte precursor cell proliferation and differentiation, and influence the development of fat tissues of the chicken (Burt *et al*., 1994; Marie *et al*., 2000). The TGF-β3 mRNA was detected in cultured chicken embryo cardiac myocytes and in heart and muscle tissues of developing chicken embryo (Jakow-lew *et al*., 1991, 1994). All these results suggest that TGF-β family genes regulate muscle development in vivo. In the study of Li *et al*. (2003), the TGF-β3 polymorphism in broilers crossed with Leghorn was associated with traits of growth and body composition, such as body weight, breast muscle weight, shank weight, abdominal fat and spleen weight. Also, the TGF-β3 gene was implicated in having a role in determining growth traits in yellow meat-type chicken (Jin *et al*., 2013). Hosnedlova *et al*. (2020) concluded that the TGF-β3 gene is associated with growth traits in broilers.

Table (2) Relationship of genotype of TGF-β3 gene with body weight (gm) in male. Mean ± standard error

Body weight	Genotype		
	AA (No. = 50)	CA (No. = 13)	CC (No. = 12)
Week 4	361.92 ± 6.29 a	368.07 ± 14.31 a	362.41 ± 14.81 a
Week 5	377.18 ± 6.38 a	384.23 ± 15.32 a	378.75 ± 15.24 a
Week 6	399.52 ± 6.69 a	406.76 ± 15.35 a	400.41 ± 15.71 a
Week 7	443.72 ± 7.25 a	450.46 ± 15.16 a	446.33 ± 16.68 a
Week 8	516.38 ± 7.74 a	526 ± 15.41 a	519.41 ± 17.69 a
Week 9	623.06 ± 8.08 a	633.15 ± 16.15 a	628.33 ± 17.92 a
Week 10	756.60 ± 8.57 a	765.69 ± 16.87 a	762 ± 18.05 a
Week 11	924.64 ± 7.26 c	998.69 ± 9.87 a	963.08 ± 8.71 b
Week 12	1092.48 ± 6.83 c	1185.30 ± 9.86 a	1142.58 ± 8.21 b
Week 13	1194.58 ± 7.48 c	1297.92 ± 10.21 a	1249.75 ± 7.66 b
Small different letters in the same raw denoted that significant differences at a level ($p<0.05$).			

Conclusions

Iraqi chickens are polymorphic in the TGF-β3 BsrI locus. The A allele frequency is higher. At the same time, the CA genotype having a positive effect on body weight could be considered for selection strategies of the birds as a parent to the next generations.

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