Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 3903 – 3917
Received 05 March 2021; Accepted 01 April 2021.

Effect of TGF-β3 Gene Polymorphism on some Blood Parameters in Local Chickens

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Abstract

Iraqi native chickens are noted for their ability to tolerate a wide variety of infectious diseases as well as high temperatures. The basic physiological routes, 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 chiefly important. However, the present study attempts to identify associations between physiological parameters and polymorphism of TGF-β3 gene in local chicken. Seventy five female birds were used in this study. A single nucleotide polymorphism was identified at 9361 position in the exons 3-6 region of TGF-β3 by using PCR-RFLP and DNA sequencing technique. The restriction enzyme BsrI has been used to detect the target region (1078 bp) in the TGF-β3 gene. The genotypic frequencies were 57.33, 24 and 18.67% for AA and CA and CC genotypes respectively. While, the allele frequency of genes A and C were 0.69 and 0.31%, respectively. Generally, during the last period of rearing we demonstrated a more positive correlation ($p>0.05$) among blood values such as RBC, Hb, WBC, H/L ratio, total protein and gamma globulin with CA genotype. In conclusion, the TGF-β3 gene could be used as a candidate gene of QTL for improving physiological traits in local chickens.

Keywords: Chicken, TGF-β3 gene, Molecular marker, Blood parameters.

Introduction

Local chickens are considered to be highly resistant to local diseases and stressful environments also require the lowest management practices, survive with fewer nutrition levels, and can endure extreme conditions (Mwacharo *et al*., 2013).

Among the genes that are involved with fitness traits, the transforming growth factor-beta subfamily (TGF-β) is one of the most important groups (Li *et al*., 2003; Enayati

and Rahimi-Mianji, 2009). The transforming growth factor-beta belongs to a large family of multifunctional growth factors that play a pivotal role in all tissues of the body for embryo and adult (Li *et al*., 2003; Wu and Hill, 2009; El-Tahawy and Abdel-Rahman, 2020). The TGF-β3 gene maps to chromosome 5 (Abasht *et al*., 2006; Wang *et al*., 2012). Proliferation, differentiation, motility, adhesion, migration, apoptosis, and immune response of epithelial, endothelial, hematopoietic, neural, and mesenchymal cells are already stimulated or inhibited by polypeptides from the TGF-β family. (Carlson *et al*., 2010; Zhang, 2012; Cooley *et al*., 2014). These roles participate in the control of normal tissue homeostasis or pathological condition (Javelaud and Mauviel, 2007; Teixeira *et al*., 2020; Poniatowski *et al*., 2015). Therefore, TGF-β genes influence many different cell types such as myogenesis, chondrogenesis, osteogenesis, hematopoiesis, epithelial cell differentiation, and adipogenesis (Massague *et al*., 1986; Roberts and Sporn, 1990; Burt and Law, 1994; Roark and Greer, 1994; Wall and Hogan, 1994).

At the same time, Ornithologists used the ratio of heterophils and lymphocytes (H/L) as a tool to monitor immune function in birds (Davis *et al*., 2008; Abdulwahid, 2015). Heterophils and monocytes in chickens are capable of phagocytosis. Heterophils are one of the first phagocytes to accumulate in the infected tissue during acute inflammatory reactions to infectious and non-infectious causes (Potter *et al*., 2016).

Immunological characteristics including antibody titers have been shown to be heritable in poultry (Yonash *et al*., 2001; Kaiser *et al*., 2002), suggesting that loci or genes related to immune or disease tolerance traits could be found. TGF-β3 gene polymorphism has also been related to bacterial resistance in chickens, such as *Salmonella enteritidis* (Kramer *et al*., 2003; Malek and Lamont 2003; Tohidi *et al*., 2012; Tohidi *et al*., 2013). In addition, the chicken antibody response (Zhou *et al*., 2002). Furthermore, there is a link between the TGF-β3 gene polymorphism and broiler chicken mortality rates (Ye *et al*., 2006). As a result, the identities of the genes or regulatory elements responsible for the inferred characteristics are unknown (Li *et al*., 2010). Candidate genes, on the other hand, are often assessed using DNA sequence variations such as single nucleotide polymorphisms (SNPs) and short tandem repeats (Abdalhag *et al.*, 2015). Therefore, the objective of the current study was to evaluate associations between TGF-β3 gene polymorphism and traits of blood.

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Materials and Methods

Study design

This study was carried out at a poultry farm, College of Veterinary Medicine/University of Baghdad during the period from 7/3 to 6/5/2019. A total of seventy five Iraqi native chicks (females) at age 28 days old were brought from the poultry research station (Abu Ghraib)/State Board for Agricultural Researches/Ministry of Agriculture. All birds were numbered by metal figures fixed (installed) on the wing pad and randomly divided in the pens. Birds were maintained according to the principle of animal welfare.

Molecular analysis

One ml of blood was collected at age 90 days old from the brachial vein of all birds. The samples were put in EDTA tubes kept in the freezer (-20 Cº) 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 Cº for 5 minutes; followed by 30 cycles of denaturation at 95 Cº for 30 seconds, annealing at 54 Cº for 30 seconds, extension at 72 Cº for 30 seconds and then final extension at 72 Cº for 7 minutes. PCR-RFLP assay including PCR products 20 µl were digested with 1 µl of BsrI restriction enzyme at 65 Cº for 1 hour. Restriction pattern were visualized under UV illumination in a 1.5% agarose gel electrophoresis stained with ethidium bromide. As well as, 20 µl of PCR products were sent to a company (Macrogene, Korea) to perform sequencing technique.

Physiological traits

In fact, two ml of blood were collected at ages 60 and 90 days old from the brachial vein of all birds. Generally, one ml of blood samples were put in EDTA tubes kept cooled for measurement of blood picture by using an automated hematology analyzer system (Mythic, Switzerland). Also, another one ml of blood samples were put in a plain plastic (without anticoagulant) tube. The blood was allowed to clot and centrifuged for 10 minutes at 3000

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rpm to obtain on serum which was stored in a deep freeze (-20 Cº). Serum total protein was measured by using a kit (Biosystem, Spain) and a spectrophotometer system. While, serum protein fractions were measured by using a kit (Hellabio, Greece) and an electrophoresis system.

Statistical analysis

The Statistical Analysis System-SAS (2012) program was used to effect of TGF-β2 gene genotype on the production parameters. Least square means (General Linear Model procedure) and Duncan's (1955) Multiple Range test was used to significant compare between means, 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 and Weinberg low; (Edwards, 2008).

Results and Discussion

DNA extraction

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

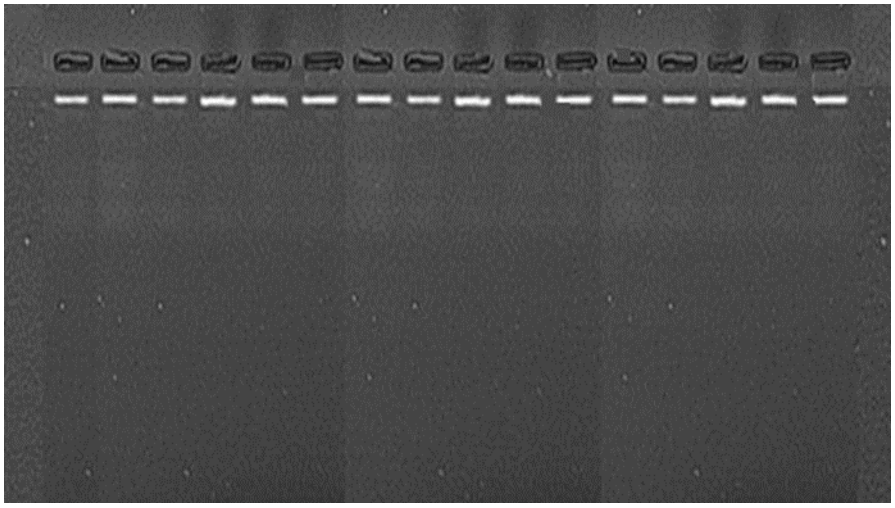

Figure (1): Genomic DNA in 1.5% agarose gel

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 result agree with Malek and Lamont 2003 and Tohidi *et al*., 2013.

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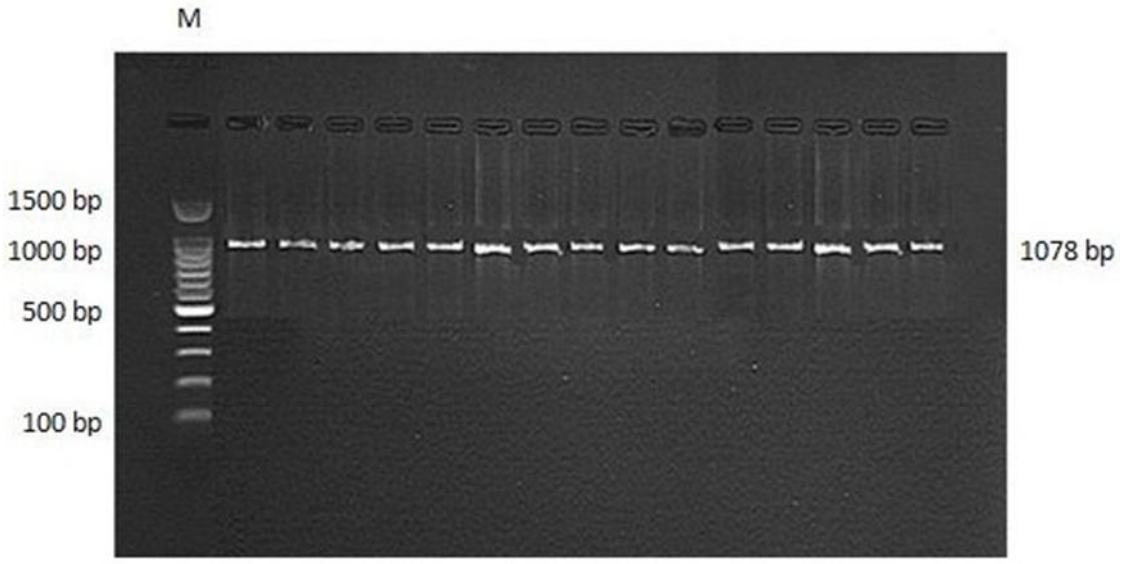

Figure (2) Amplification TGF-β3 gene in 1.5% agarose gel. M= 100 bp ladder.

PCR-RFLP assay

The PCR products which underwent restriction digestion with BsrI enzyme (ACTGGN/ and /NCCAGT) to detect SNP C>A in the exons 3-6 of TGF-β3 gene and it was able to cut at this location only when SNP is present (when transformed C to A). The following fragment sizing patterns were discovered by agarose gel electrophoresis Figure (3).

1. Wild type AA: BsrI was cut the sequence to show four fragments in agarose gel electrophoresis (500 bp, 404 bp, 124 bp and 50 bp).
2. Heterozygous CA: BsrI was cut the sequence to show six fragments in agarose gel electrophoresis (500 bp, 404 bp, 301 bp, 199 bp, 124 bp and 50 bp).
3. Homozygote CC: BsrI was cut the sequence to show five fragments in agarose gel electrophoresis (404 bp, 301 bp, 199 bp, 124 bp and 50 bp).

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The current result agree with Malek and Lamont 2003; Tohidi *et al*., 2013.

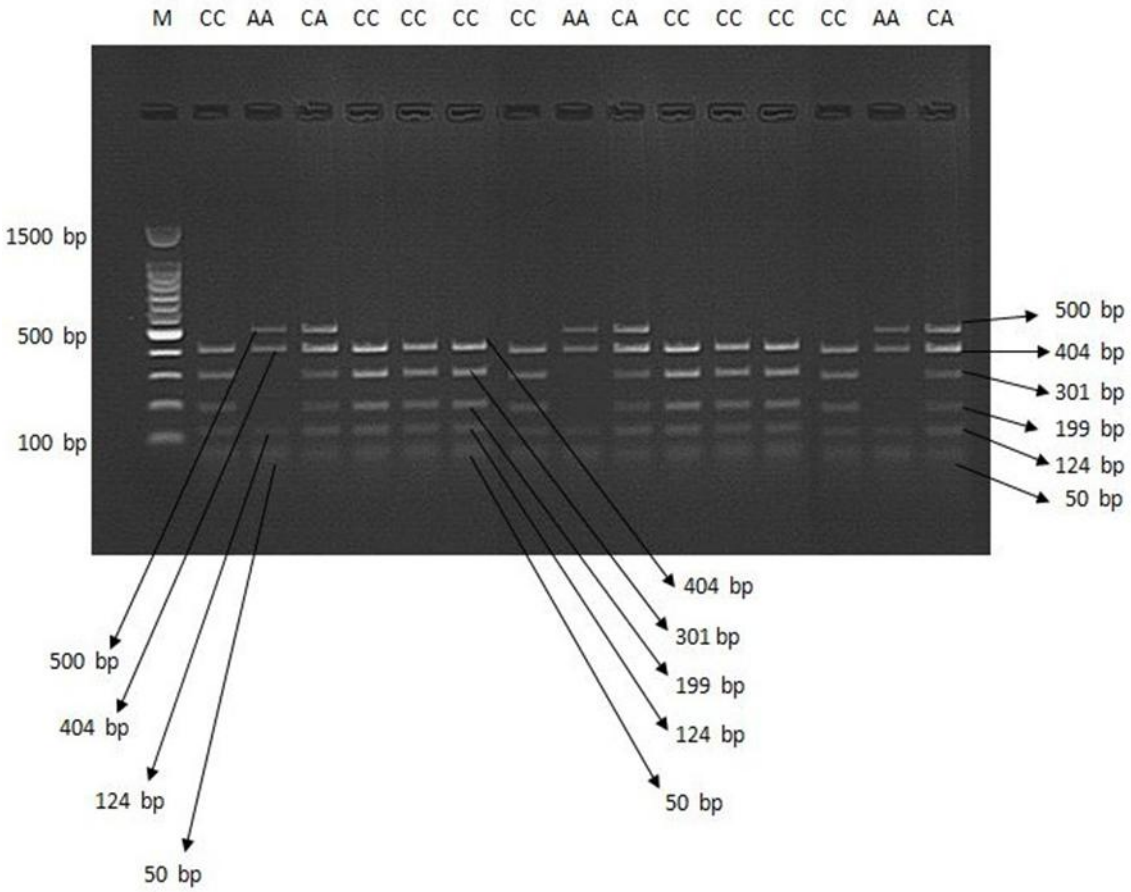

Figure (3): BsrI restriction pattern of fragment of TGF-β3 by PCR-RFLP on 1.5 % agarose gel. M= 100 bp ladder; CC, CA, AA = genotype.

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 found at position 9361 bp in the exon 3-6 Figure (4).

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

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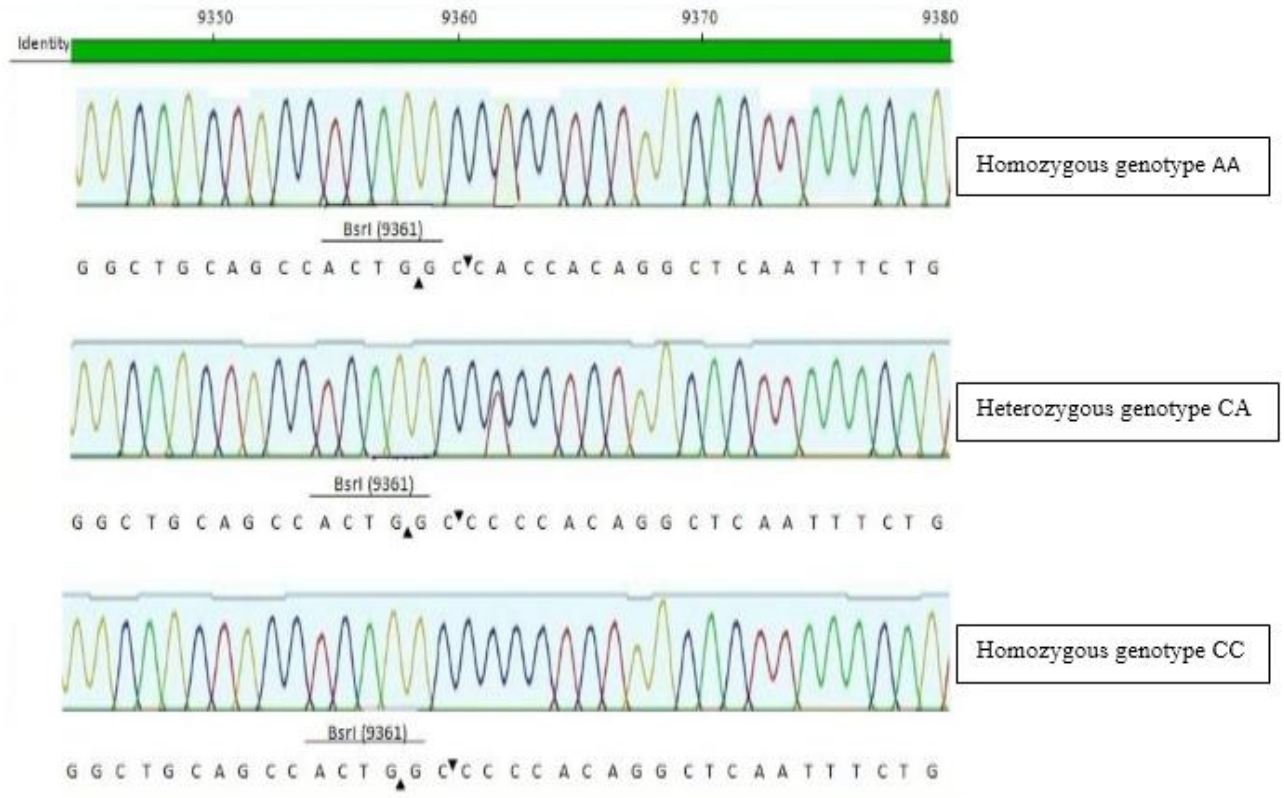

Figure (4): Sequence analysis of TGF-β3 gene

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 non-significant variations ($p>0.01$) in the rate of three genotypes. The genotype AA had the highest percentage (57.33%), followed by genotype CA (24%), and genotype CC had the lowest percentage (18.67%), and the allele frequency of A was dominant on allele frequency of C that reached 0.69 and 0.31 as A and C allele respectively, As the law of (Hardy Weinberg equilibrium). The Chi-Square analysis revealed that the association of BsrI allelic pattern with strain is non-significant.

Based on the results of this study, it can be concluded that differences in the level of single nucleotide polymorphism 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).

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

Genotype	Number	Percentage (%)
AA (Wild)	43	57.33
CA (Heterozygous)	18	24
CC (Mutant)	14	18.67
Total	75	
Chi-Square (X2)	8.129 *	
Allele	Frequency	
A	0.69	
C	0.31	
* (p>0.01).		

Effect of TGF-β3 gene on hematological values

The results of Table (2) show non-significant differences among the three genotypes of TGF-β3 gene with blood values at the age of 60 days. As well as, PCV, hetrophils, lymphocytes, basophils, esonophils and monocytes at the age of 90 days. While, there were significant differences in RBC, Hb, WBC and H/L ratio in the age 90 days by CA genotype that was dominant on the other genotypes 3.76 10^6/µL, 10.40 mg/dl, 25.11 103/µL and 0.35 followed by CC genotype 3.41 10^6/µL, 9.96 mg/dl, 24 103/µL and 0.37 then AA genotype 3.06 10^6/µL, 9.17 mg/dl, 22.69 10^3/µL and 0.37 respectively.

Current research suggests that the correlation between the TGF-β polymorphism and hematological values is caused by linkage disequilibrium between the mutations and another mutation located in the TGF-β or another related gene that is specifically involved in the regulation of these phenotypic traitsAs an organ that has a very high amount of T and B lymphocytes, the spleen plays a crucial role in immune response and disease resistance. Because the RNA for TGF-β3 is expressed in the spleen of embryo, newly hatched chicks, and adults (Jakowlew *et al*., 1997), they may play a role in development of the spleen in the chicken. Thus, the results regarding the effects of TGF-β3 genotype on spleen weight indicated possible identification of this gene as quantitative trait loci (QTL) of spleen weight.

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Table (2) Relationship of genotype of TGF-β3 gene with hematological values at different ages. Mean ± standard error

Parameters	Age	Genotype		
		AA (No. = 43)	CA (No. = 18)	CC (No. = 14)
RBC (10^6/µL)	60 days	2.26 ± 0.14 a	2.33 ± 0.13 a	2.17 ± 0.09 a
	90 days	3.06 ± 0.09 b	3.76 ± 0.22 a	3.41 ± 0.15 ab
pcv (%)	60 days	26.28 ± 0.36 a	26.66 ± 0.37 a	26.32 ± 0.26 a
	90 days	28.97 ± 0.27 a	30.14 ± 0.55 a	29.83 ± 0.47 a
Hb (mg/dl)	60 days	8.65 ± 0.22 a	8.56 ± 0.25 a	8.35 ± 0.17 a
	90 days	9.17 ± 0.15 b	10.40 ± 0.32 a	9.96 ± 0.27 a
WBC (103/µL)	60 days	19.88 ± 0.20 a	20.33 ± 0.31 a	20 ± 0.34 a
	90 days	22.69 ± 0.19 c	25.11 ± 0.21 a	24 ± 0.34 b
Hetrophils (%)	60 days	23.20 ± 0.29 a	22.65 ± 0.45 a	22.85 ± 0.50 a
	90 days	24.41 ± 0.21 a	23.44 ± 0.40 a	24.15 ± 0.43 a
Lymphocytes (%)	60 days	63.63 ± 0.27 a	64.53 ± 0.47 a	64.38 ± 0.52 a
	90 days	65.06 ± 0.25 a	66.11 ± 0.42 a	65 ± 0.44 a
H/L ratio	60 days	0.36 ± 0.005 a	0.35 ± 0.009 a	0.35 ± 0.010 a
	90 days	0.37 ± 0.003 b	0.35 ± 0.007 a	0.37 ± 0.007 ab
Basophils (%)	60 days	2.25 ± 0.14 a	1.61 ± 0.23 a	1.71 ± 0.32 a
	90 days	1 ± 0.11 a	1 ± 0.16 a	1 ± 0.20 a
Esonophils (%)	60 days	3.13 ± 0.15 a	2.88 ± 0.29 a	2.92 ± 0.28 a
	90 days	1.58 ± 0.16 a	1.40 ± 0.21 a	1.78 ± 0.26 a
Monocytes	60 days	7.79 ± 0.23 a	8.33 ± 0.34 a	8.14 ± 0.32 a

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(%)	90 days	7.95 ± 0.18 a	8.05 ± 0.31 a	8.07 ± 0.38 a
Small different letters in the same raw denoted that significant differences at a level ($p<0.05$).				

Effect of TGF-β3 gene on serum protein profile

The results of Table (3) show non-significant differences among the three genotypes of TGF-β3 gene with all protein profile at the age of 60 days. As well as, albumin, alpha 1, alpha 2 and beta globulins at the age of 90 days. While, there were significant differences in total protein and gamma globulin at the age of 90 days by CA genotype that was dominant on the other genotypes 42.03, 12.64, followed by CC genotype 38.59, 10.95 then AA genotype 36.98 and 10 g/l respectively.

Proteins make up most of the compounds in your serum. In turn, proteins are the building blocks of all cells and body tissues. Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins. Hepatocytes are responsible for the majority of blood protein synthesis and secretion (Zhou *et al*., 2016). Immunoglobulins, which are formed by the immune system, are the main exceptions. (Eckersall, 2008; Jackson and Elsawa, 2015). Immune cells such as lymphocytes (T and B cells), natural killer cells, macrophages, dendritic cells, and granulocytes respond to TGF-β with effects on proliferation, survival, activation, and differentiation (Li, *et al*., 2006; Flavell *et al*., 2010; Sanjabi *et al*., 2017.

Based on our results, it can be concluded that the increased concentrations of total protein and gamma globulins in the blood sera of birds may confirm the immunoregulation effect of the TGF-β3 gene.

Table (3) Relationship of genotype of TGF-β3 gene with serum protein profile (g/l) at different ages. Mean ± standard error

Parameters	Genotype			
	Age	AA (No. = 43)	CA (No. = 18)	CC (No. = 14)
Total protein	60 days	32.20 ± 0.26 a	32.31 ± 0.37 a	31.42 ± 0.37 a
	90 days	36.98 ± 0.30 c	42.03 ± 0.21 a	38.59 ± 0.45 b
	60 days	14.10 ± 0.08 a	14.13 ± 0.13 a	13.87 ± 0.12 a

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Albumin	90 days	15.96 ± 0.08 a	16.30 ± 0.16 a	15.80 ± 0.10 a
Alpha 1 globulin	60 days	4.06 ± 0.03 a	4.31 ± 0.04 a	3.53 ± 0.07 a
	90 days	5.03 ± 0.03 a	5.15 ± 0.05 a	5.76 ± 0.04 a
Alpha 2 globulin	60 days	1.48 ± 0.02 a	1.49 ± 0.03 a	1.53 ± 0.04 a
	90 days	2.09 ± 0.01 a	2.09 ± 0.02 a	2.11 ± 0.02 a
Beta globulin	60 days	2.52 ± 0.03 a	2.46 ± 0.04 a	2.50 ± 0.05 a
	90 days	3.90 ± 0.03 a	3.85 ± 0.03 a	3.97 ± 0.05 a
Gamma globulin	60 days	10.04 ± 0.06 a	9.92 ± 0.10 a	9.99 ± 0.14 a
	90 days	10 ± 0.13 c	12.64 ± 0.18 a	10.95 ± 0.12 b
Small different letters in the same raw denoted that significant differences at a level ($p<0.05$).				

Conclusion

This information can be used to recommend SNP as a candidate for Marker Assisted Selection. These findings can be applied in the chicken selection strategy to obtain better physiological performance.

Acknowledgments

The authors would like to express their gratitude to all of the workers at the Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Iraq, for their continued support, advice, effort, and encouragement.

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