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CLINICAL STUDY WITH INVESTIGATION OF POLYMORPHISM TGF-β4 GENE IN IRAQI CHICKEN

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Abstract

In this study, we are breeding Sixty chicks, at the age of one day for six lines from local chicken (White, white naked neck, Brown, Brown naked neck, Black, Barred). Six chicks were taken from each of the six local chicken lines for a total of thirty six chicks studied. And the breeding was in a closed system under ideal environment (ventilation, humidity, temperature) and all chicks with the recommended diet**.** At the age of thirty days, we take the blood wing vein from each bird about (3cc) blood, and the blood was put in tubes containing anticoagulants (EDTA), these tubes were placed in a cool box and taken to the laboratory for the purpose of DNA extraction and DNA was extracted by a thermometer system and used PCR technique**.** The result showed that two genotype for gene, there are FF genotype (wild) , BF genotype (hetrozyous).There was significantly affect ($p<0.05$) of the TGF-β4 gene polymorphism on the mean weekly live body weights at the age of (5th,6the,7th,8th) weeks, the genotype BF recorder highest mean in that age, There was significantly affect ($p<0.05$) of the TGF-β4 gene polymorphism on the mean weekly body weight gain at the age of (6the,7th,8th) weeks,the genotype BF recorder highest mean in that age. There was significantly affect ($p<0.05$) on feed intake and feed conversion ratio the genotype FF the highest recordered.
***Keywords*:** Polymorphism, TGF-β4 Gene, Iraqi chicken

Introduction

Chicken is a major source of protein rich diet which continuous as high as 54% protein per 100 gm (Bhalla *et al.,* 2015). Poultry is an important source of eggs, meat and income in the rural areas. In the developing countries, they have been branded as a tool for poverty alleviation and food security (Magothe and Kahi, 2010). The generation of a high quality draft sequence for the genome of chicken (*Gallus gallus*) is an important advance (Shadan, 2013; Dhyaa *et al*., 2014). Chickens are good models for studying the genetic basis of phenotypic traits, because of the extensive diversity among domestic chicken selected for different purpose . Monogenic traits are well-studied (Pisenti *et al*., 2001; Dodgson and Romanov, 2004; Nicholas, 2003). TGF-β4 gene effected on chicken growth and development and role in processes such as mayogenesis, chondrogenesis, osteogenesis, hematoposis, and depogenesis (Cogburn *et al*., 2000).

Materials and Methods

A total of Sixty Iraqi chicks ranged of one day old with for six lines from local chicken (White, white naked neck, Brown, Brown naked neck, Black, Barred). Blood sample (5 ml) was gatheredaseptically from jugular vein of chicks into tubes containing anticoagulants (0.5 M EDTA) for each animal. The total DNA produced by the standard protocol by intron kit (Korea) procedure. Two conserved primers, forward: 5- GGG GTC TTC AAG CTG AGC GT - 3 and reverse: 5- TTG GCA ATG CTC TGC ATG TC-3. Thermal cycling included: Denaturation at 95 °C for 3 min, followed by 37 cycles of 94 °C for 30s, 55°C for 30s and 72 °C for 35s with final incubation at 72 °C for 7 min. Polymerase chain reaction products were extracted using electrophoresis at 2% agarose gel with the visualized by contact with ultraviolet light (302nm). Chi square used for statistical analysis (SAS, 2012).

Result and Discussion

Relationship of the Genotype of TGF-β4 with weekly body weight in local chickens

In this results show significant differences ($P<0.05$) among the two genotypes of TGF-β4 in weekly body weight of local chicken on the 5th, 6th, 7th, 8th weeks by BF genotype comparing with FF genotype. (287.51, 364.19, 444.27, 509.08) gm for the 5th, 6th, 7th, 8th weeks. While there were non – significant differences with the body weight on 1st day old and weeks (1st, 2nd, 3th and 4th) table (1).

Table 1 : Relationship of the Genotype of TGF-β4 gene with body weight.

Body weight: Weeks	Mean ± SE of body weight (gm)		Level of sig.
	FF	BF	
Wt. at hatching	32.66 ± 1.18	32.81 ± 1.38	NS
Week 1	58.64 ± 0.97	58.95 ± 0.98	NS
Week 2	92.73 ± 1.72	92.89 ± 1.99	NS
Week 3	151.48 ± 3.01	152.08 ± 3.05	NS
Week 4	208.16 ± 3.99	212.44 ± 4.35	NS
Week 5	277.93 ± 5.28 b	287.51 ± 6.21 a	*
Week 6	340.57 ± 5.41 b	364.19 ± 8.04 a	*
Week 7	417.69 ± 6.78 b	444.27 ± 9.87 a	*
Week 8	486.34 ± 8.06	509.08 ± 11.90	*

* ($P<0.05$), S: Significant
Means having with the different letters in same row differed significantly.

In table (1), it have been shown that weekly body weight of the chicken with allel BF was significantly higher as compared with chicken body weight that carry FF allel at fifth, sixth, seventh and eighth week of their life *($p<0.05$).* this give an indication that chicken with allel BF is economically better that chicken with allel FF. Many approaches may be used to improve chicken lines for meat

production; Chambers (1990) has published an extensive review on that issue. They have demonstrated the efficiency of selection to improve broiler body weight. Chambers JR *et al*. (1981). The chicken TGF-β subfamily consists of four currently identified members: TGF-β1, TGF-β2, TGF-β3, and TGF- β4 (Burt, *et al*., 1992). Given the role that TGF-β superfamily genes play in growth and development, they are logical targets for investigation as candidate genes for economically important allels in chickens. Chicken TGF-β 4 mRNA were detected in cells of all germ layers at Stage 10 of embryo development (Jakowlew *et al*., 1992). Growth is a comprehensive reflection of development of various parts of a chicken body, and its final expression is the result of interaction among genetic, nutritional, and environmental factors. Growth is under complex genetic control, and un-covering the molecular mechanisms of growth will con-tribute to more efficient selection for growth in broiler chickens (Scanes *et al*., 1984). A previous study heve been showed that TGF-β genes may be important in growth rate, tibia and shank development and growth, breast muscle weight and yield, amount of abdominal fat, and spleen weight in chickens growing to market weight. The TGF-β genes are, therefore, potential markers for use in molecular marker-assisted selection programs. Also they found that TGF-β4 birds had the major effect on bone (tibia) mineral content and tibia length also they found that TGF-β4 can be used as a candidate gene of quantitative triat locus (QTL) useful for selecting for bone strength and length (Li H, *et al*., 2003), this study did not focus on the effect of different triat of chicken with TGF-4 on body weight but it study other parameters have an effect on general health of chicken, these parameters may have indirect influence on the weight of the chicken, this finding is synansm with the present study finding in which the the increase in the weight of chicken have been detected in both triat but one triat of TGF-4 had a better effect on weight gain then other triat.

Relationship of the Genotype of TGF-β4 with body weight gain

The results of table (2) show significant differences (P<0.05) among the two genotype of TGF-β4 in body weight gain of local chicken on the 6^{th},7^{th},8^{th} weeks by BF genotype comparing with FF genotype (76.68 , 89.08 , 99.81) gm for the 6^{th}, 7^{th}, and 8^{th} weeks. While there were non-significant differences with weekly body weight gain on 1^{st} day old and weeks (1^{st}, 2^{nd}, 3^{th}, 4^{th} and 5^{th}).

Table 2 : Relationship of the Genotype of TGF-β4 with body weight.

Weight gain: Weeks	Mean ± SE of weight gain (gm)		Level of sig.
	FF	BF	
Week 1	25.98 ± 1.46	26.13 ± 1.59	NS
Week 2	34.09 ± 1.12	33.93 ± 1.43	NS
Week 3	58.74 ± 1.58	59.19 ± 1.47	NS
Week 4	56.68 ± 1.56	60.36 ± 2.04	NS
Week 5	69.76 ± 2.47	75.06 ± 1.87	NS
Week 6	62.64 ± 6.02 b	76.68 ± 8.71 a	*
Week 7	77.12 ± 3.20	89.08 ± 3.56	*
Week 8	81.64 ± 3.52	99.81 ± 4.14	*
Total gain	463.68 ± 8.17 b	486.26 ± 12.04 a	*

* (P<0.05), S: Significant
Means having with the different letters in same row differed significantly.

At the sametime, linking the finding in table (2) with table (1) made the picture even more clear about the influence of diifrent triat on weight of chicken, in which the table (2) chicken with allel BF have a significant weight gain as compare with chicken with allel FF in sixth, seventh and eighth week of their life

Relationship of the Genotype of TGF-β4 gene with Total feed intake and FCR

The result of table (3) show significant differences (P<0.05) in total feed intake the genotype FF that reach to 1735.78 Kg comparative with BF genotype. In FCR (Feed conversion Rito) the genotype FF that reach to 3.826 kg comparative with BF genotype.

Table 3 : Relationship of the Genotype of TGF-β4 gene with Total feed intake and FCR.

Parameters	Mean ± SE		Level of sig.
	FF	BF	
Total feed intake (kg)	1735.78±82.48 a	1594.04±73.02 b	*
Mean of FCR (kg feed/kg meat)	3.826 ± 0.41 b	3.347 ± 0.57 a	*

* (P<0.05), S: Significant
Means having with the different letters in same row differed significantly.

In table (3), the food consumption and feed conversion ratio of chicken with allel BF of TGF-4 was significantly lower when compare with the food consumption of chicken with allel FF of TGF-4 *(p<0.05).*the relationship between TGF-β4 gene and/or its allel with food consumption and feet conversion ratio is not achieved yet, so there is no previous information about this relationship. The goals in feeding poultry differ between different classes of poultry. In general, for poultry raised to provide meat, such as broilers, the aim is to produce the maximum body weight gain for the minimum cost of feed while controlling the amount of fat on the body. For egg laying birds, the aim is to maximise egg production for the minimum cost of feed while controlling the egg size and egg quality (Henry LC,2017). Feed cost accounts for the greatest proportion of broiler production variable costs, having a direct impact on farm productivity, and therefore, on its profitability. Production efficiency is significantly and negatively affected when feed nutritional levels are lower than the broilers' requirements (Kamran *et al*., 2008). On the other hand, supplying diets with nutrient levels above the requirements improves live performance, but may result in economic losses due the higher cost of those diets (Moosavi *et al*., 2011). The Economics of feed conversion ratio (FCR), also known as feed conversion efficiency, is one of the most important principles for a chicken breeding. The cost of chicken feed claims over half of the total budget for chicken farm. Consequently, it is very important for a chicken farmer to get the optimum performing feed for the most economical price, so that there is inverse relationship between feed conversion ration and the cost benefit for raising and breeding of chicken in other word lower feed conversion ratio is best financial benefit (Martins JMS, *et al*., 2016). in table (3) of the present study chicken with allel BF have significant lower food consumption with significant lower feed conversion ratio as compared with FF allel of TGF-4, this give an indication that chicken with BF allel is economically better that FF allel. The analysis of TGF-β4

gene polymorphism was carried out using PCR method. Genomic DNA of goat was successfully amplified by pair of primer that covers entire coding sequence of TGF-β4 gene. Genomic DNA of white blood cells was also used for amplification of TGF-β4 gene using PCR specific primers. The amplified fragment which is yielded of single band of the desired product of TGF-β4 gene with a molecular weight of 240 base pair appeared sharp in agarose gel through Gel electrophoreses technique and loaded with (100-1000bp) DNA ladder (figure 1).

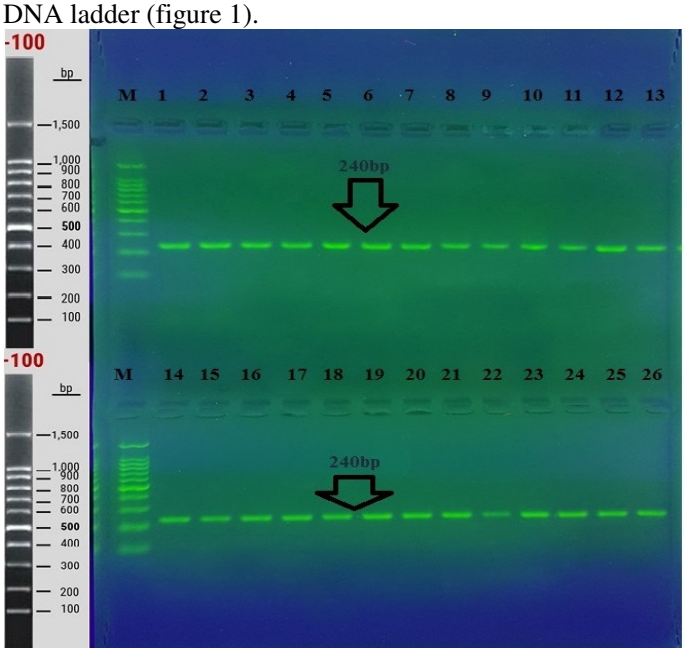

Fig. 1 : PCR product the band 240 bp for TGF-β4 gene. The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

The PCR product was digested with restriction endonucleases and the genetic polymorphisms were investigated by PCR-RFL. There are two cleavage sites of genotypes *BF* (249 bp, 173 bp) within the amplification fragment of one genotypes *FF* (240 bp) were detected in local Iraqi chicken (Figure3).

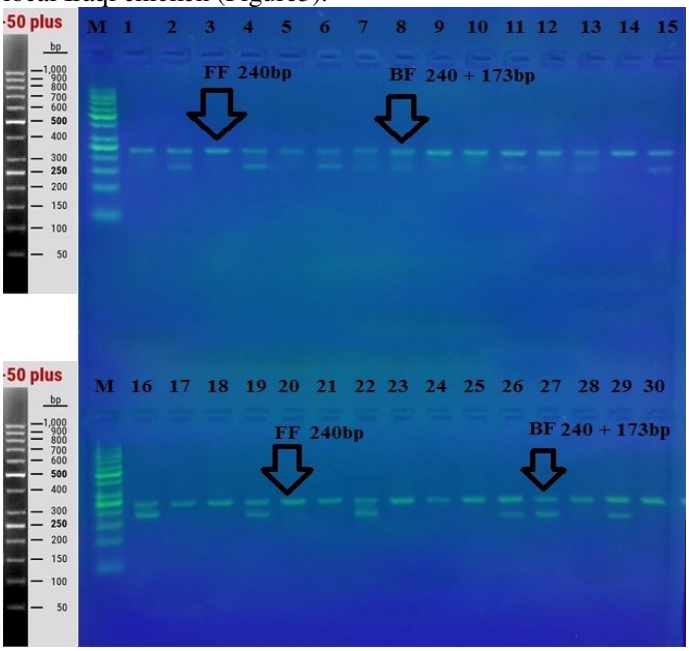

Fig. 3: Electrophoresis pattern of PCR product digested with *MboII* restriction enzyme (2% agarose gel) in chicken. Lane's homozygous: FF genotype (single strand 240 bp); Lane's heterozygous: BF genotype (two strand 240,173 bp). With, M: DNA molecular marker 100 bp size and by red stain in the gel

References

Bhalla, A.S.; Alatafi, A.K.; Singh, M.K.; Lal, R. and Parvangada, A. (2015). Genetic diversity of Himalayan Region Chicken through SSR Molecular Markers. International Journal Science and Research, 4(9): 1311-1314.

Burt, D.W. and Paton, I.R. (1992). Evolutionary origins of the transforming growth factor-beta gene family. DNA Cell Biol. 11: 497–510.

Chambers, J.R.; Gavora, J.S. and Fortin, A. (1981). Genetic changes in meat-type chickens in the last twenty years. Canadian Journal of Animal Science; 61: 555-563.

Cogburn, L.A.; Burnside, J. and Scanes, C.G. (2000). Physiology of growth and development. Pages 635–656 in Sturkie's Avian Physiology. 5th ed. G.C. Whittow, ed. Academic Press, San Diego.

Dhyaa, Ab.A.; Ali, F.R.; Azhar, S.K. and Myson, A.A. (2014). Comparative anatomical and histological features of the kidney in Harrier (*Circus aueroginosus*), Chicken (*Gallus domesticus*) and Mallard duck (*Anas platyrhynchos*) The Iraqi Journal of Veterinary Medicine, 38(1): 017 – 003.

Dodgson, J.B. and Romanov, M.N. (2004). Use of chicken models for the analysis of human disease. In: Dracopoli, N. C., editors. Current Protocols in Human Genetics. Hoboken: John Wiley & Sons, Pp. 15.5.1-11.

Henry, L.C. (2017). Diet energy and feed intake in chickens. Animal Feed Science and Technology; 233: 13-21.

Jakowlew, S.B.; Ciment, G.; Tuan, R.S.; Sporn, M.B. and Roberts, A.B. (1992). Pattern of expression of transforming growth factor-beta 4 mRNA and protein in the developing chicken embryo. Dev. Dyn. 195: 276–289.

Kamran, Z.; Sarwar, M.; Nisa, M.; Nadeem, M.A.; Mahmood, S.; Babar, M.E. and Ahmed, S. (2008). Effect of low-protein diets having constant energy to protein ratio on performance and carcass characteristics of broiler chickens from one to thirty five days of age. Poultry Science; 87: 468-474.

Li, H.; Deeb, N.; Zhou, H.; Mitchell, D.A.; Ashwell, C.M. and Lamont, S.J. (2003). Chicken Quantitative Allel Loci for Growth and Body Composition Associated with Transforming Growth Factor-β Genes1. Poultry Science: 82(3): 347-56.

Magothe and Kahi (2010). Indigenous chicken improvement in Kenya; past efforts and future prospects. Ministry of Livestock and Fisheries Development (MOLFD) (1996-2007). Various Animal Production Annual Reports.

Martins, J.M.S.; Carvalho, C.M.C.; Litz, F.H.; Silveira, M.M.; Moraes, C.A.; Silva, M.C.A. (2016). Productive and Economic Performance of Broiler Chickens Subjected to Different Nutritional Plans. Brazilian Journal of Poultry Science, 18(2): 209-216.

Moosavi, M.; Eslami, M.; Chaji, M. and Boujarpour, M. (2011). Economic value of diets with different levels of energy and protein with constant ratio on broiler chickens. Journal of Animal and Veterinary Advances; 10: 709-711.

Nicholas, F.W. (2003). Online Mendelian Inheritance in Animals (OMIA): a comparative knowledge base of genetic disorders and other familial traits is non-laboratory animals. Nucleic Acids Res. 31: 275-277.

Pisenti, J.M. (2001). Avian genetic resources at risk: an assessment and proposal for conservation of genetic stocks in the USA and Canada. Avian Poultry Biology Rev. 12: 1-102.

SAS Global Forum, (2012).

Scanes, C.G.; Harvey, S.; Marsh, J.A. and King, D.B. (1984). Hormones and growth in poultry. Poult. Sci., 63: 2062–2074.

Shadan, H.A. (2013). Prevalence of Blood Parasites in Local Chickens in Qaradagh District, Sulaimani – Iraq, The Iraqi journal of Veterinary Medicine, 37(1): 17–21.