Al-Muthanna J. For Agric Sci Print ISSN: 2226-4086 Online ISSN:2572-5149
Vol. 11 , Issue.Supplement 01. 2024
https://muthjas.mu.edu.iq/ http://doi.org/10.52113/mjas04/11.1/21

Effect of Insulin-Like Growth Factor (IGF-2) Gene Polymorphisms on Blood Cells Performance in Broiler Chickens

Ali S. Al-Hassani[1], D. H. Al-Hassani[2] and I. A. Abdul-Hassan[3]

[1,2] Department of Animal Production, College Agriculture Engineering Science, University of Baghdad, Baghdad, Iraq.
[3] Genetic Engineering and Biotechnology Institute for Postgraduate Study, University of Baghdad, Baghdad, Iraq.

[1]E-mail: ali@coagri.uobaghdad.edu.iq

Received on 2/01/2024 Accepted on 20/3/2023 Published on 1/4/2024

Abstract. The purpose of this study was to examine the genetic makeup of insulin-like growth factor-2 (IGF-2) and its impact on the various components of blood cells. This study utilized a total of 300 broiler chicks, aged one day, from two different strains: 150 chicks from the Cobb 500 strain and 150 chicks from the Hubbard F-15 strain. Genomic material was isolated from avian blood samples, and the constituent elements of the blood were analyzed. The blood components were cytologically examined and their genotypes for insulin-like growth factor-2 (IGF-2) were determined using PCR-RFLP. The study observed a notable rise ($p\leq0.05$) in the level of Hb in the bloodstream of females ($p\leq0.05$) with the genotypes TT and CC. The findings indicated a statistically significant increase ($p\leq0.05$) in the PCV% of females with the CC genotype compared to those with the TT and TC genotypes. The findings also indicated that there were no statistically significant variations ($p\leq0.05$) in the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MHCV) across the different genotypes. The IGF2 gene incorporates variables related to ethnicity and sex. The number of Heterophil cells in the offspring of the Cobb500 strain with the TC genotype was significantly higher ($p\leq0.05$) compared to the TT and CC models. Additionally, there was a significant increase ($p\leq0.05$) in the number of Heterophil cells in males with the TC genotype compared to the TT and CC models, while this increase was not observed in other cases. The IGF2 gene genotypes exhibit notable variations in both lymphocyte count and the H/L ratio. Multiple genotypes of the IGF-2 insulin-like growth factor gene were discovered to have a notable impact on the quantity of eosinophil cells in the Hubbard F-15 strain. This effect was observed in males of all genotypes involved in the experiment. The experiment revealed a notable rise in the count of basophil cells among females with the CC genotype compared to individuals with other genotypes. However, there were no significant variations in the number of basophil cells ($p\leq0.05$) among individuals with other genotypes or between the two strains. The results did not show a statistically significant difference in the number of monocyte cells or in any of the IGF2 genotypes, taking into account the influence of strain and sex.

Keywords. Poultry, PCR-RFLP, DNA and mRNA.

1. Introduction

The IGF-2 gene in chickens regulates the growth and maturation of satellite cells, skeletal muscles, body composition, fat accumulation, and metabolic function. According to reference [1], the pituitary growth hormone stimulates the production of IGF-2 in the liver. The liver is the main source of IGF-2 production, however it is also produced by the pituitary gland, brain, ovary, spleen, and muscle. Growth hormone (GH) induces the synthesis of insulin-like growth factor 2 (IGF-2) by binding to the growth hormone receptor (GHR) and initiating the activation of the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway. The PI3K-Akt, motor, and MAPK pathways are

the pathways responsible for IGF-2-induced muscle cell hyperplasia and hypertrophy [2, 3]. IGF-2 demonstrates several metabolic roles [4]. The given data represents the coordinates as [5, 6]. The production of IGF-2 by the liver, which has an impact on tissues through the endocrine system, is controlled by growth hormone and diet [4]. IGF-2 promotes the development and maturation of organisms after they are born. The IGFII gene, situated on chromosome 1, harbors quantitative trait loci (QTLs) responsible for governing growth [5, 6]. IGF-II is essential for chicken growth [7, 8]. A strong link exists between the performance of laying hens and broilers and the 5' flanking region of single nucleotide polymorphisms (SNPs) in the promoter region of the insulin-like growth factor I (IGFI) gene. Gouda and Essawy conducted a study to assess the influence of IGF-II gene polymorphism on chicken characteristics [4]. The study established a correlation between the IGFII gene polymorphism and many parameters, including body mass, sexual maturity, egg weight, and egg number, in two distinct populations. Furthermore, it revealed a correlation between the IGF-2 gene polymorphism and the body weights of Cobb 500 and Hubbard F-15 broiler chickens. The IGF-2 gene possesses the capacity to exert an impact on the growth of broiler chickens. The study conducted by [9] revealed that the recently identified IGF-2 plays a vital role in the metabolism of glucose, lipids, and proteins. Protein makes up adipose tissue, skeletal muscle, and the hepatic organ. IGF-2 reduces blood glucose levels and improves the body's sensitivity to insulin, hence boosting the control of glucose levels in the body [10]. IGF-2 suppresses the process of producing glucose in the kidneys, which could result in a decrease in blood glucose levels [11]. Using candidate genes in chicken breeding provides the potential to improve genetic features. Certain genes has the capacity to augment industrial performance. Chickens have a multitude of possible genes, including one called IGF-2. The number 12 is denoted by the numeral [12]. The structure of the IGF-2 gene does not exhibit any association with the variety observed in chicken phenotypes (12). Further research is required to examine the correlation between IGF-2 polymorphisms and chicken performance. This study examined the influence of IGF-2 polymorphisms on blood cell function in Hubbard F-15 and Cobb500 broiler lines.

2. Material and Methods

The Department of Animal Production in the College of Agricultural Engineering Sciences at the University of Baghdad conducted this research. This study utilized Cobb 500 and Hubbard F-15 broilers. Each broiler chick that is hatched for commercial purposes is fitted with a wing band. We restricted the broilers to a designated area within an experimental habitat. The document presents a thorough record of the sleep and food schedules of the participants, together with the level of attention they received in accordance with the prescribed standards for their specific breeds. At 49 days old, we obtained blood from the wing veins and then divided it into two equal halves. The initial portion was employed for serum analysis, whilst the latter portion was dedicated to DNA extraction. The DNA purification technique utilized a genomic DNA purification kit from Delta Bio Techs, situated in Iraq. We utilized a 260/280 nm optical density (OD) ratio to evaluate the integrity of the sample and identify any protein impurities in a one-liter volume of extracted DNA, with the aim of quantifying the DNA content (ng/L). The DNA ratio of 260/280 fell within the range of 1.7 to 1.9. We perform laboratory tests on serum samples to ascertain the concentrations of glucose, triglycerides, hepatic enzymes, and LDL cholesterol. The DNA was extracted utilizing a saline solution. The primers IGF2-F and IGF2-R were used to amplify a 1146-base-pair fragment of the IGF2 gene. The primers IGF2-F and IGF2-R shown satisfactory performance in prior experiments [12]. A 25-liter volume was used for the polymerase chain reaction (PCR), consisting of the following components: The components required for the experiment are as follows: 50 mM of dATP, dTTP, dCTP, and dGTP each; 0.5 mM of each primer; 2.5 L of 10X PCR buffer; 2 mM of magnesium chloride; 2.5 U of Taq DNA polymerase; and 50 ng of extracted DNA. The experiment involved 35 amplification cycles, each comprising a 1-minute denaturation step at 94°C, followed by a 3-minute extension step at 72°C, and concluding with a 5-minute extension step at 72°C. The PCR results were visualized by illuminating the gel with UV light. The PCR products were fragmented with

HinfI. A total of 15 liters of the solution, consisting of 5 liters of PCR product, 5 units of Hinf I endonuclease, and 1.5 liters of Hinf I buffer, were digested. A time period of 2 hours at a temperature of 37 °C. We applied the broken-down DNA fragments to electrophoresis on a gel made of 1.5% agarose and observed them using UV trans-illumination. We utilized PopGene32 1.23 [13] to compute allelic and genotypic frequencies, as well as observed and anticipated heterozygosity. We utilized the PopGene 32 software to evaluate the status of the Hardy-Weinberg equilibrium. The figures represent the ratio of each allele. A total of 1146 PCR products, each consisting of a pair of base pairs, were produced. A solitary band was identified in every chicken blood sample through the utilization of genomic DNA. Following the acquisition of the PCR results, we proceeded to do RFLP analysis. IGF2 PCR products were cleaved using HinfI to yield RFLP patterns. The T allele was found in 73.3% of the population, whereas the C allele was found in 26.7%, leading to the existence of three genotypes.

.

3. Results and Discussion

3.1. Hemoglobin Concentration and PCV%

The results of this study indicate that there was no association between blood Hb levels and IGF-2 genotypes. The variations in blood hemoglobin levels between different breeds did not have an impact on the outcomes of the investigation. Blood hemoglobin concentrations exhibited a statistically significant disparity (P≥0.05) between males and females with the TT and CC genotypes (Table 1) observed in chickens. Nevertheless, the hemoglobin concentration in the blood of broiler chicks was determined to be 8.25 mg/dl [1]. Researchers have suggested that IGF-2 has a substantial impact on determining the normal level of hemoglobin in the blood [14]. Through the reduction of Fe concentration [11], it has been demonstrated that IGF-2 has the ability to directly decrease Hb levels. Additionally, by stimulating the IGF-2 receptor in skeletal muscle, IGF-2 can enhance the effect of insulin on Hb transportation. IGF-2's ability to reduce and increase sensitivity to insulin may have a beneficial effect on the regulation of Hb homeostasis [10]. Furthermore, there was no notable impact on the PCV% across various genotypes, breeds, and genders. According to this study, there was a statistically significant difference (P≥0.05) observed in males with genotype CC compared to those with genotypes TT and TC.

Table 1. The effect of IGF-2 gene polymorphism on Fresh Blood Parameters and compare this effect, Cobb versus Hubbard and male versus female.(Means±SE).

IGF-2 genotype	Breed			Sex			Total
	Cobb	Hubbard	*p*	Male	Female	*p*	
	Hb(gm./100 ml) (means ± SE)						
TT	8.22±0.12	8.22±0.13	*NS*	8.20±0.13	8.02±0.12	*0.05*	8.20±0.13
TC	8.25±0.13	8.24±0.13	*NS*	8.22±0.12	8.25±0.12	*NS*	8.25±0.12
CC	8.20±0.12	8.21±0.14	*NS*	8.25±0.12	9.03±0.13	*0.05*	8.22±0.13
p	*NS*	*NS*		*NS*	*0.05*		*NS*
	PCV (%) (means ± SE)						
TT	32.16±0.62	32.15±0.60	*NS*	32.18±0.62	32.15±0.62	*NS*	32.16±0.60
TC	32.15±0.60	32.14±0.62	*NS*	32.15±0.60	32.14±0.62	*NS*	32.15±0.62
CC	32.18±0.65	32.18±0.65	*NS*	32.17±0.65	35.17±0.65	*0.05*	32.18±0.65
p	*NS*	*NS*		*NS*	*0.05*		*NS*

IGF-2 =insulin-like growth factor-2; SE= standard error; p= probability; 0.05= significant at p≤0.05; NS= no significant.* = significant at 0.05 level between Cobb and Hubbard and between male and female within each genotype.

3.2. MCV, MCH Levels and MCHC

The IGF-2 genotype did not have any impact on the levels of MCV and MCH in the bloodstream, as seen in Table 2. This study found that gene polymorphisms, breeds, and gender did not have any impact on MCV and MCH levels. The MCV value

ranged from 140 to 141 femtometres, while the MCH value was 32 pictograms. The blood of birds is primarily constituted of MCV. [15], Peebles and colleagues [16] observe that there are decreases in MCV management in meat hens as they age. However, in Hubbard F-15 broilers, all genotypes have exhibited significantly elevated levels of blood MCV and MCH (P≤0.05) compared to other Cobb500 genotypes and MCHV%. Researchers observed a significant increase (P≤ 0.05) in the MCV and MCH concentrations of Hubbard F-15 broilers compared to Cobb500 broilers. The TC and CC genotypes of Cobb 500 and Hubbard F-15 had no impact on MCV and MCH levels in the blood, according to the researchers' findings. There was no variance observed in the concentrations of MCV and MCH in male broiler genotypes. Similarly, there was also no variation observed in female broiler genotypes across all genotypes (P ≤0.05). The MCHV% levels in the blood were not substantially different among individuals with TT, TC, and CC genotypes, regardless of gender [17]. In a study conducted by Hassan and colleagues, it was found that Hubbard F-15 and female broilers bearing the C allele had lower levels of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in their blood compared to those that did not have the allele. The results of a study on the TC genotype of broiler chickens have been reported [18].

Table 2. The effect of IGF-2 gene polymorphism on Fresh Blood Parameters and compare this effect, Cobb versus Hubbard and male versus female.

IGF-2 genotype	Breed			Sex			Total
	Cobb	Hubbard	*p*	Male	Female	*p*	
MCV (Femtometre) (means ± SE)							
TT	140±0.15 7	140±0.15	*NS*	140±0.15	141±0.12	*NS*	140±0.15
TC	141±0.15	140±0.15	*NS*	140±0.12	140±0.16	*NS*	140±0.15
CC	140±0.16	140±0.16	*NS*	140±0.17	140±0.16	*NS*	140±0.16
p	*NS*	*NS*		*NS*	*NS*		*NS*
MCH (Pictogram) (means ± SE)							
TT	32±0.20	32±0.23	*NS*	32±0.20	32±0.22	*NS*	32±0.22
TC	32±0.22	32±0.20	*NS*	32±0.22	32±0.20	*NS*	32±0.20
CC	32±0.20	32±0.20	*NS*	32±0.20	32±0.20	*NS*	32±0.30
p	*NS*	*NS*		*NS*	*NS*		*NS*
MHCH (%) (means ± SE)							
TT	30.18±0.65	30.12±0.62	*NS*	30.16±0.65	30.18±0.65	*NS*	30.16±0.65
TC	30.15±0.60	30.15±0.65	*NS*	30.15±0.60	30.17±0.60	*NS*	30.15±0.60
CC	30.15±0.63	30.18±0.63	*NS*	30.15±0.63	30.15±0.65	*NS*	30.15±0.65
p	*NS*	*NS*		*NS*	*NS*		*NS*

IGF-2 =insulin-like growth factor-2; SE= standard error; p= probability; 0.05= significant at p≤0.05; NS= no significant. * = significant at 0.05 level between Cobb and Hubbard and between male and female within each genotype.

Table 3. The effect of IGF-2 gene polymorphism on Fresh Blood Parameters and compare this effect, Cobb versus Hubbard and male versus female.

IGF-2 genotype	Breed			Sex			Total
	Cobb	Hubbard	*p*	Male	Female	*p*	
Heterophil ($\times 10^3$/µL) (means ± SE)							
TT	3.32±2.25 *	3.16±2.24	*0.05*	3.16±2.25	3.16±2.25	*NS*	3.16±2.25
TC	3.15±2.20	3.15±2.20	*NS*	3.23±2.25*	3.15±2.20	*0.05*	3.15±2.20
CC	3.12±2.22	3.13±2.20	*NS*	3.12±2.20	3.12±2.20	*NS*	3.12±2.20
p	*0.05*	*NS*		*0.05*	*NS*		*NS*
Lymphocyte ($\times 10^3$/µL) (means ± SE)							
TT	5.42±2.75	5.45±2.73	*NS*	5.45±2.75	5.45±2.75	*NS*	5.45±2.75
TC	5.40±2.70	5.42±2.70	*NS*	5.42±2.70	5.43±2.70	*NS*	5.42±2.70
CC	5.43±2.72	5.40±2.73	*NS*	5.43±2.70	5.40±2.72	*NS*	5.43±2.72
p	*NS*	*NS*		*NS*	*NS*		*NS*
H/L Ratio (means ±							

TT	0.61±0.08	0.58±0.08	*NS*	0.58±0.09	0.58±0.08	*NS*	0.58±0.08
TC	0.58±0.07a	0.58±0.07	*NS*	0.60±0.07	0.58±0.07	*NS*	0.58±0.07
CC	0.57±0.07a	0.58±0.07	*NS*	0.57±0.07	0.57±0.07	*NS*	0.57±0.07
p	*NS*	*NS*		*NS*	*NS*		*NS*

IGF-2 =insulin-like growth factor-2; SE= standard error; p= probability; 0.05= significant at p≤0.05; NS= no significant. * = significant at 0.05 level between Cobb and Hubbard and between male and female within each genotype.

Table 4. The effect of IGF-2 gene polymorphism on Fresh Blood Parameters and compare this effect, Cobb versus Hubbard and male versus female.

IGF2 genotype	Breed			Sex			Total
	Cobb	Hubbard	*p*	Male	Female	*p*	
	Eosinophil ($\times10^3$/µL) (means ± SE)						
TT	0.76±0.20	1.20±0.20*	*0.05*	0.75±0.22	1.20±0.22	*0.05*	0.85±0.22
TC	0.80±0.20	1.12±0.22	*0.05*	0.82±0.20	1.12±0.20	*0.05*	0.82±0.20
CC	0.83±0.22	1.08±0.22	*0.05*	0.82±0.22	1.08±0.22	*0.05*	0.82±0.20
p	*NS*	*0.05*		*NS*	*0.05*		*NS*
	Basophil ($\times10^3$/µL) (means ± SE)						
TT	3.20±0.42	3.26±0.42	*NS*	3.23±0.43	3.20±0.42	*NS*	3.20±0.42
TC	3.22±0.40	3.20±0.40	*NS*	3.20±0.43	3.22±0.40	*NS*	3.22±0.40
CC	3.22±0.40	3.23±0.43	*NS*	3.25±0.40	3.89±0.40*	*0.05*	3.25±0.40
p	*NS*	*NS*		*NS*	*NS*		*NS*
	Monocyte ($\times10^3$/µL) (means ± SE)						
TT	1.16±0.32	1.13±0.32	*NS*	1.15±0.32	1.16±0.32	*NS*	1.16±0.32
TC	1.15±0.30	1.14±0.30	*NS*	1.12±0.30	1.15±0.30	*NS*	1.15±0.30
CC	1.16±0.33	1.13±0.33	*NS*	1.17±0.33	1.16±0.33	*NS*	1.16±0.33
p	*NS*	*NS*		*NS*	*NS*		*NS*

IGF-2 =insulin-like growth factor-2; SE= standard error; p= probability; 0.05= significant at p≤0.05; NS= no significant. * = significant at 0.05 level between Cobb and Hubbard and between male and female within each genotype.

3.3. Blood Hetrophil, Lymphocyte and H/L Ratio

From (table 3) that shows the levels of Hetrophil and Lymphocyte cells and also H/L Ratio. The result shown there were no significant effect (P≤0.05) in TC and CC genotypes between Hubbard F-15 and Cobb 500 breeds .But there were significant effect (P≤0.05) in TT genotype higher than other genotypes in Hitrophil cells count [19]. About the sex, the results shown Significant increase (P≤0.05) in female at TC genotype compared to others in hetrophil cells count [20]. Results shown no significant differences (P≤0.05) in lymphocyte account between all genotypes in Hubbard F-15 vs Cobb500 breed, but also results shown significant difference (P≤0.05) on female in CC genotype compare to TT and Tc genotype [21]. Results also no significant differences (P≤0.05) in H/L Ratio among all genotypes and between males and females [22].

3.4. Eosinophil, Basophil and Monocyte Counts

According the results (table 4) there was significant increase (P≤0.05) in Eosinophil cells count in Hubbard F-15 breed compeer to Cobb500 in all IGF2 genotypes under study [23-27]. Also, there was significant increase (P≤0.05) in Eosinophil cells count in females than males in all genotypes. There were no significant differences (P≤0.05) between genotypes in Basophil cells account in Hubbard F-15 and Cob 500 breed [28]. But there was significant increase (P≤0.05) in Basophil Cells count in CC genotype of Females compeer to TT and TC genotypes [29-31]. The results show no significan

difference (P≤0.05) in Monocyte Cells count between all IGF2 genotypes including breeds and sex. The results were accepted with the results of [32].

Conclusion

Females with genotypes TT and CC had significantly higher Hb levels (p≤0.05). PCV% was greater (p≤0.05) in CC genotype females than TT and TC genotype females. MCV, MCH, and MHCV for each genotype did not vary significantly (p≤0.05). IGF2 gene race/sex factors. Offspring revealed a significant increase in Hitrophil cells (p≤0.05) in the Cobb500 strain of the TC genotype compared to the TT and CC models, as well as in the TC genotype of males compared to the TT and CC models, but it was not found in the TT and CC models. IGF2 genotypes affect lymphocyte count and H/L ratio. Multiple genotypes of the IGF-2 insulin-like growth factor gene affected the number of Hubbard F-15 strain Eosinophil cells and males of all genotypes in the experiment. In the experiment, the number of Basophil cells in females of the CC genotype was significantly higher than in the other genotypes and strains (p≤0.05). The quantity of Monocyte cells and IGF2 genotypes, including strain and sex, did not vary significantly.

References

[1] Boschiero C, EC Jorge, K Ninov, K Nones, MF do Rosario, LL Coutinho, MC Ledur, DW Burt and ASA Moura.2013. Association of IGF-1 and KDM5A polymorphisms with performance, fitness and carcass traits in chickens. Journal of Applied Genetic. 54:103-112.

[2] Chatterjee R, RP Sharma, TK Bhattacharya, M Niranjan and BL. Reddy.2010 Microsatellite variability and its relationship with growth, egg production, and immune competence traits in chickens. Biochemical Genetics. 48:71-82.

[3] Clemmons DR.2007. Modifying IGF1 activity: an approach to treat endocrine disorders, atherosclerosis and cancer. Nature Reviews Drug Discovery. 6:821-833.18.

[4] Dong H, L Zeng, D Duan, H Zhang, Y Wang, W Li and H. Lin .2010 .Growth hormone and two forms of insulin-like growth factors I in the giant grouper (Epinephelus lanceolatus): molecular cloning and characterization of tissue distribution. Fish Physiology and Biochemistry. 36:201-212.

[5] Girbau M, JA Gomez, MA Lesniak and F. Pablo .1987.Insulin and insulin-like growth factor I both stimulate metabolism, growth and differentiation in the postneurula chick embryo. Endocrinology. 121:1477-1482.

[6] Gouda EM, GS. Essawy.2010 Polymorphism of insulin-like growth factor I gene among chicken breeds in Egypt. Zeitschrift für Naturforschung. 65:284-288.

[7] Hassan H, H Guo, H Jin and M. 2007. Galal Relation between abdominal fat and serum cholesterol, triglycerides, and lipoprotein concentrations in chicken breeds. Turkish Journal of Veterinary and Animal Sciences. 31(6):375-379.

[8] Hayes BJ, PJ Bowman, AC Chamberlain and ME. Goddard.2008. Genomic selection in dairy cattle: progress and challenges. Journal of Dairy Science. 92:1313.

[9] Hu SY, CC Tai, YH Li and JL. Wu .2012. Progranulin compensates for blocked IGF-1 signaling to promote myotube hypertrophy in C2C12 myoblasts via the PI3K/Akt/mTOR pathway. FEBS Lett. 586:3485- 3492.

[10] Kadlec J, B Hosnedlova, V Rehout, J Cítek, L Vecerek and L. Hanusova .2011. Insulin-like growth factor-I gene polymorphism and its association with growth and slaughter characteristics in broiler chickens. Journal of Agrobiology. 28(2):157-63.

[11] Kanački Z, S Stojanović , G Uśćebrka and D. Žikić .2012 the development pattern of IGF-1 (insulin- like growth factor-1) protein expression in breast muscle of broiler chickens. Biotechnology in Animal Husbandry. 28(4):797-805.

[12] Kanbur NO, O Derman and E. Kinik.2005. The relationships between pubertal development, IGF-1 axis, and bone formation in healthy adolescents. Journal of Bone and Mineral Metabolism. 23:76-83.

[13] Richmond W. Clinical Chemistry. 1973; 19:1350-1356.

[14] Scanes CG.2009. Perspectives on the endocrinology of poultry growth and metabolism. General and Comparative Endocrinology. 163:24-32.

[15] Zhou H, CM Evock, JP Mcmurtry, CM Ashwell and SJ.Lamont .2007.Genome-wide linkage analysis to identify chromosomal regions affecting

phenotypic traits in the chicken. IV. Metabolic traits. Poultry Science. 86: 267-276.

[16] Zanou N, Gailly P.2013. Skeletal muscle hypertrophy and regeneration: Interplay between the myogenic regulatory factors (MRFs) and insulin-like growth factors (IGFs) pathways. Cellular and Molecular Life Sciences. 70:4117-4130.

[17] Trinder P. 1969.Determination of blood glucose using an oxidase-peroxidase system with a non- carcinogenic chromogen. Journal of Clinical Pathology. 22(2):158-161.

[18] Tang S, D Sun , J Ou ,Y Zhang ,G Xu , Y. Zhang .2010 .Evaluation of the IGFs (IGF1 and IGF2) genes as candidates for growth, body measurement, carcass, and reproduction traits in Beijing You and Silkie chickens.Animal Biotechnology. 21:104-113.

[19] Sidney PG, Barnard R.1973. Improved manual spectro- photometric procedure for determination of serum triglycerides. Clinical Chemistry. 19:1077-1078.

[20] Scanes CG.2008. Perspectives on analytical techniques and standardization. Poultry Science. 87(11):2175-2177.

[21] SAS. 2010. Statistical Analysis System, Systems for Windows.SAS Institute Inc.

[22] Sambrook J, Russell DW.2001. Molecular Cloning: A Laboratory Manual. 3rd Edn, Cold Spring Harbor Laboratory Press, New York. 49-56.

[23] Peebles ED, JD Cheaney, JD Brakea, CR Boyle and MA.Latour.1997. Effects of added dietary lard on body weight and serum glucose and low density lipoprotein cholesterol in random bred broiler chickens. Poultry Science. 76:29-36.

[24] McMurtry JP, JL Francis and Z. Upton .1997. Insulin-like growth factors in poultry. Domestic Animal Endocrinology. 14(4):199-229.

[25] Landau D,R Eshet, A Troib,Y Gurman,Y Chen,R Rabkin and Y. Segev.2009. Increased renal Akt/m TOR and MAPK signaling in type I diabetes in the absence of IGF type 1 receptor activation. Endocrine. 36:126-134.

[26] Krasnodebska A, Koncicki A .2000. Physiological values of selected serum biochemical indices in broiler chickens. Medycyna Wet. 56:456-460.

[27] Kabir G, M Mosaraf, F Omar, H Naimul, H Zahid, N Quamrun , M Sultana , A Mohammad and A. Liaquat .2010. Associations of serum free IGF-1 and IGFBP-1 with insulin sensitivity in impaired glucose tolerance (IGT). International Journal of Diabetes Mellitus. 2:144-147.

[28] Junnila RK, EO List , DE Berryman , JW Murrey and JJ. Kopchick .2013.The GH/IGF-1 axis in ageing and longevity. Nature Reviews Endocrinology. 9:366-376.

[29] Carolyn S, P Lam, C Ming, M Sean, Y Qiong, M Lisa, X Vanessa, S Radwan, M Holly, P Xuyang, B Dougla and S. Ramachandran.2010. Circulating insulin-like growth factor-1 and its binding protein-3. Arteriosclerosis Thrombosis and Vascular Biology. 30:1479-1484.

[30] Bulut Z, E Kurar, Y Ozsensoy, M Nizamlioglu, M Garip, A Yilmaz, T Caglayan, S Dere, V Kurtoglu and M. Dogan .2013. Determination of chromosomal regions affecting body weight and egg production in Denizli White Leghorn F2 populations. Eurasian Journal of Veterinary Sciences. 29:30-38.

[31] Bergmeyer HU. 1985 .Methods of enzymatic analysis, Deerfield Beach. VCH Publishers. 6:191-199. [32] Al-Hassani AS, D.H Al-Hassani and I.A. AL-Hasan .2015 .Association of Insulin-Like Growth Factor-1 Gene Polymorphism at 279 Position of the 5'UTR Region with Body Weight Traits in Broiler Chicken. Asian Journal of Poultry Science.9; 213-222.