INSULIN LIKE GROWTH FACTOR (IGF-2) GENE POLYMORPHISMS INFLUENCES CERTAIN BIOCHEMICAL PARAMETERS IN BROILER CHICKENS

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ABSTACT

This study aimed to investigate genetic variations of the IGF-2 gene and how these polymorphisms affect different biochemical markers was conducted in this study. Broiler chicks of 300 days of age were analyzed in this research (150 Cobb500 and 150 Hubbard F-15). Each bird's blood was used to get its unique DNA sequence. Multiple biochemical features were connected to IGF-2 genotypes found using PCR-RFLP. If you're looking for an easy way to raise your chickens' cholesterol levels, go no further than Cobb500 chickens with the "TT" genotype (p≤0.05). Hubbard F-15 broilers with the TT genotype exhibited substantially higher levels of LDL (p≤0.05) than those with the TC and CC genotypes. The LDL levels of Hubbard F-15 broilers were substantially greater than those of Cobb500 broilers (p ≤0.05). In male TC-type broilers, LDL levels were considerably (p ≤0.05) greater than those in the TC-type female broilers. The ALT enzyme activity of broilers with the TC genotype was substantially greater than that of broilers with the TT genotype (p≤0.05). Cobb500 broilers with the CC genotype had substantially lower AST enzyme activity than Cobb500 broilers with the TT or TC genotypes (p≤0.05). There was a significant difference (p≤ 0.05) in AST enzyme activity between the Hubbard F-15 broiler genotypes, TC and TT, and CC genotypes. IGF-2 gene polymorphisms were shown to have a greater impact on ALT and AST enzyme activity than other gene variations.

Keywords: poultry, PCR-RFLP, DNA and mRNA

تأثير تعدد الطرز الجينية لعامل النمو الشبيه بالأنسولين (IGF-2) على بعض صفات الدم الكيميائية والحيوية في فروج اللحم.

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المستخلص

تم إجراء دراسة لطرز جين عامل النمو الشبيه بالانسولين-2 IGF-2 وكيفية تأثيرها على صفات الدم البايوكيميائية. استخدم في هذا البحث 300 فرخ من افراخ فروج اللحم بعمر يوم واحد من سلالتي (150 كوب 500 و 150 هوبارد إف 15). تم استخراج الحمض النووي من دم الطيور واجراء الفحص لصفات الدمم الكيميو حيوية . تم ربط صفات الدم الكيمياحيوية بالطرز الجينية لعامل النمو الشبيه بالانسولين -2 IGF-2 باستخدام PCR-RFLP .وجد من خلال الدراسة ارتفاع معنوي(p≤0.05) في مستويات الكوليسترول في الدم لسلالة Cobb500 في الطراز الوراثي TT. أظهرت الدجاج من سلالة Hubbard F-15 ذات التركيب الوراثي TT مستويات أعلى من LDL وبصورة معنوية (p≤0.05) من تلك التي تحتوي على االطرز الجينية TC و CC وكانت مستويات LDL في فروج اللحم من سلالة Hubbard F-15 أكبر بكثير من تلك الموجودة في فروج اللحم من سلالة Cobb500 عند مستوى معنوية (p ≤0.05). فروج اللحم من الطراز الوراثي TC ، كانت مستويات LDL أكبر بكثير (p ≤0.05) من تلك الموجودة في الطرز الاخرى . كان نشاط إنزيم ALT لفروج اللحم ذات التركيب الوراثي TC أكبر بكثير من نشاط التركيب الوراثي TT عند مستوى معنوية (p≤0.05). كان لفروج اللحم من سلالة Cobb500 ذات الطراز الوراثي CC نشاط إنزيم AST أقل بكثير من دجاج التسمين Cobb500 مع الأنماط الجينية TT أوTC عند مستوى معنوية (p≤0.05). وكان هناك فرق معنوي (p≤ 0.05) في نشاط إنزيم AST بين الطرز الجينية لفروج اللحم من سلالة Hubbard F-15 TC و TT و CC . وتبين أن الطرز المتعددة لجين عامل النمو الشبيه بالانسولين IGF-2 لها تأثير كبير في نشاط إنزيمات ALT و AST اكثر الصفات الاخرى.

الكلمات المفتاحية: الدواجن، PCR-RFLP , mRNA,DNA .

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INTRODUCTION

One of the primary regulators of chicken satellite cell proliferation and skeletal muscle hypertrophy (1) as well as of body composition, growth, fat deposition, and metabolic activity (2) has been shown to be IGF-2 in chickens. Growth hormone action in the pituitary triggers the liver to create IGF-2 (3). The pituitary, brain, ovary, spleen, and muscle all produce IGF-2, despite the liver being the primary source of IGF-2. By binding to the GHR and activating the JAK-STAT pathway, GH stimulates IGF-2 synthesis. The PI3K-Akt pathway, the motor system, and the MAPK pathway all play a role in IGF-2-induced muscle cell hyperplasia and hypertrophy (6, 7). A member of the pre-proinsulin polypeptide hormone family, insulin-like growth factor-2 (IGF-2) has a wide range of metabolic functions (8). (9)(10) Growth hormone and food regulate the liver's production of IGF-2, which has endocrinological effects on the tissues it targets (8). During the period of postnatal growth, IGF-2 plays a key role. Growth-controlling QTLs have been found in a linkage region around the IGFII gene on chromosome 1 (9, 10). IGF-II has been shown in studies (11, 12) to be critical to the proper development of chickens. Research in the 5' flanking area (the promoter region for IGFI) of SNPs in laying hens and broilers has showed a high correlation between the two traits' performance. To find out whether the IGF-II gene polymorphism in chicken breeds has any effect on the development of poultry features, researchers Gouda and Essawy looked into the matter (8). (9) Tang et al. (13) examined two Chinese populations to discover the relationship between IGFII gene polymorphism and body mass, sexual maturity, egg weight, and egg quantity (Beijing and Silkies). Al-Hassani et al. (14) found that IGF-2 gene polymorphism was linked with broiler body weights in Cobb500 and Hubbard F-15 broilers. And this suggests that the IGF-2 gene might be a potential for influencing broiler chicken growth. For the metabolism of carbs, fats, and proteins, Kanacki et al. found that IGF-2 is required. All of the body's tissues are made up of protein, including fat, muscle, and liver. IGF-2 is thought to improve glucose homeostasis by reducing blood glucose levels and increasing insulin sensitivity (16). Clemmons found that IGF-2 inhibits renal gluconeogenesis, which might lower blood sugar levels (17). Using candidate genes in the chicken breeding program has become a powerful tool for genetic improvement. Manufacturing performance may be improved by incorporating a candidate gene into an organism's genome. There are a number of potential candidate genes in chickens, including the insulin-like growth factor-II (IGF-2) gene. (18) The association between the IGF-2 gene structure and phenotypic diversity in chicken breeds has not yet been established (18). Further investigation into the link between IGF-2 locus polymorphisms and chicken performance qualities is needed. ‘IGF-2 polymorphisms were discovered and examined in Hubbard F-15 and Cobb500 broiler lines for their effects on phenotypes of many biochemical variables as part of the present study.

METERIAL AND METHODES

This study was conducted at University of Baghdad's College of Agricultural Engineering Sciences / Animal Production Department and the University's Genetic Engineering and Biotechnology Institute. Breeds of broilers employed in this study were the Cobb500 and Hubbard F-15. Individual wing bands were attached to commercially hatched broiler chicks. In an experimental home, during this time, the broilers were kept in the same place. This includes where they slept and ate, as well as how they were treated in accordance with breed standards. Samples of blood were extracted from the wing veins at 49 days of age and split into two halves; the first half was collected in an anticoagulant-free tube to get serum, while the second half was collected in EDTA-coated tubes to extract DNA. A genomic DNA purification kit was used to clean up the DNA (Delta Bio Techs, Iraq). An optical density (OD) ratio of 260/280 nm was utilized to assess the purity of the samples to detect protein contamination in the one liter of extracted DNA that was used for DNA concentration measurement (ng/L). The allowed 260/280 DNA ratios varied from 1.7 to 1.9. Testing for blood sugar and

triglycerides, as well as liver enzymes and LDL cholesterol is done in the serum. DNA was extracted quickly using salt-extraction techniques. It was decided to employ primer pairs (IGF2-F) and (IGF2-R) to amplify a 1146 bp fragment of the IGF2 gene for the experiment. The primers (IGF2-F) and (IGF2-R) have been shown to work well in previous studies (Amills et al. 2003). A 25 l reaction volume was used to do PCR, which included the following: 50mM dATP, 50mM TTP, 50mM CTP, and 50mM GTP, 0.5mM of each primer, 2.5l of 10X PCR buffer, 2mM magnesium chloride, 2.5 U of Taq DNA polymerase, and 50ng of extracted DNA as the template. The 35 amplification cycles utilized in this experiment included one minute of denaturation at 94°C, three minutes of extension at 72°C, and one last five minutes of extension at 72°C. UV trans-illumination on a 1.5 percent agarose gel revealed the PCR results. Restrictor HinfI was used to cut the PCR products into smaller pieces. Digestive procedures were carried out in 15 l of mixed mixtures that included 5l of PCR product, 5 U Hinf I endonuclease, and 1.5 l of Hinf I buffer. It was incubated for two hours at 37 °C. A 1.5 percent agarose gel was used to separate the digested DNA fragments, which were then seen under UV trans-illumination. Allelic and genotypic frequencies, as well as actual and anticipated heterozygosity, were calculated using PopGene32 version 1.23 (23). Using PopGene 32, the Hardy-Weinberg equilibrium test was also carried out. Figures were compiled to show the proportion of each kind of allele. The 1146 bp PCR products were successfully made. Only one particular band appeared in the PCR results of all chicken blood samples that included genomic DNA recovered from chickens. As a result, RFLP analysis was carried out as soon as the PCR results were available. These RFLP patterns were created by digesting PCR products produced from the IGF2 gene with HinfI enzyme. With 73.3 percent of the population having the T allele and 26.7 percent having the C allele, there were two allele frequencies and three genotypes.

RESULTS AND DISCUSSION

Serum glucose concentrations: There was no correlation between blood glucose levels and IGF-2 genotypes, according to the results of this study. Genetic and breed differences in blood glucose levels did not affect the results of the study. Blood glucose concentrations did not differ significantly across breeds or sexes within any genotype (Table 1). Plasma glucose values of 153 mg/dl (26) were recorded for hens; however, the plasma glucose of broiler chicks was found to be 165 mg/dl (1). A significant role for IGF-2 in establishing a baseline blood glucose level was proposed by Mc Murtry and colleagues (27). By decreasing renal gluconeogenesis, Clemmons (17) showed that IGF-2 may lower glucose levels directly, and by activating the IGF-2 receptor in skeletal muscle, IGF-2 can boost insulin's impact on glucose transport. IGF-2 may have a positive impact on glucose homeostasis because of its glucose-lowering and insulin-sensitizing properties (16).

Blood triglyceride and cholesterol levels

IGF-2 gene polymorphisms, breeds, and gender had no influence on blood cholesterol levels in the present study, according to the findings (Table 1). Injecting IGF-2 (10–100 ng/embryo-1) into 2-day-old chicken embryos raised the cholesterol levels in 4-day-old embryos, according to Girbau et al. Cobb500 broilers with the TT genotype exhibited considerably greater levels of blood triglycerides (p 0.05) than their counterparts with the TC or CC genotypes (82.52 mg/dl vs. 73.12 mg/dl and 75.234 mg/dl, respectively). A blood triglyceride level difference between male Hubbard breeders and both sexes was not significant, nor was a difference between male Hubbard breeders and males of any other breed (Table 1). C allele in Cobb500 breed has been connected to lower blood lipid levels in this study. It is important to remember that genetics have a role in determining serum cholesterol and triglyceride levels. In experiments on chicken development, researchers have noticed a broad variety of outcomes (30). This may be one of the causes.

HDL and LDL levels in the bloodstream

IGF-2 genotype had no effect on HDL levels in the bloodstream. Gene polymorphisms, breeds, and gender had no effect on HDL levels in this study Total cholesterol included 35–42% HDL. Birds' blood is mostly composed of high-density lipoprotein (HDL).

(33), According to Peebles and colleagues (31), age-related declines in HDL cholesterol management were seen in meat chickens. However, in Hubbard F-15 broilers, TT genotype was shown to have substantially higher levels of serum LDL ($p \leq 0.05$) in comparison to other Cobb500 genotypes (20.22, 18.90, and 18.93, respectively). To compare Hubbard F-15 broilers' with Cobb500 broilers' serum LDL concentrations, researchers found Hubbard F-15 broilers' to be considerably higher ($p \leq 0.05$). The TC and CC genotypes of Cobb500 and Hubbard F-15 dogs were shown to have no effect on LDL levels in the blood. Male broiler genotypes showed no variation in LDL concentrations, whereas female broiler genotypes showed substantial differences ($p \leq 0.05$) in LDL concentrations (18.55 instead of 18.65, and 20.22 instead of 20.35) when compared to the two other genotypes (CC and TT). Men with the TC genotype had significantly higher levels of serum LDL (22.15 IU/L vs 15.32 IU/L) than women ($p \leq 0.05$). Serum LDL levels were not significantly different between TT and CC genotype men and women, regardless of gender. C allele-carrying Hubbard F-15 and female broilers exhibited reduced LDL levels in their blood than non-carrying ones. Males in Anka and Rugao had considerably ($p \leq 0.01$) higher levels of LDL than females in a research by Hassan and colleagues.A similar study on the TC genotype of broiler chickens has produced these results.

Table 1. For Cobb and Hubbard breeds, IGF-2 gene polymorphisms affected glucose, cholesterol, and triglyceride levels in blood at 42 days old. (Mean ±SE)

IGF-2 genotype	Breed			Sex		
	Co	Hubbard	p	M	Female	p
		glucose (mg./100ml)				
TT	143.22 ±	158.72 ±	N	154.66 ±	153.52 ±	NS
TC	152.90 ±	161.25 ±	N	156.27 ±	162.62 ±	NS
CC	165.00 ±	147.65 ±	N	157.69 ±	159.16 ±	NS
p	N	NS		N	NS	
		cholesterol (mg./100ml)				
TT	116.22 ±	123.92 ±	N	115.66 ±	116.33 ±	NS
TC	117.88 ±	123.60 ±	N	116.36 ±	116.26 ±	NS
CC	115.19 ±	124.58 ±	N	118.55 ±	117.38 ±	NS
p	N	NS		N	NS	
		triglyceride (mg./100ml)				
TT	82.52 ±	70.90 ±	N	76.85 ±	69.58 ± 4.29	NS
TC	73.12 ±	73.82 ±	N	77.62 ±	68.84 ± 4.28	NS
CC	75.34 ±	70.83 ±	N	76.80 ±	70.00 ± 4.22	NS
p	0.	NS		N	NS	

Between breeds and sexes within each genotype, and between genotypes and sexes within each genotype, there was no statistically significant difference in IGF-2 levels between genotypes. Genotypes are distinguished by the a-b letters if they vary significantly from each other at a 0.05 level of significance

Table2. At 42 days of age, the IGF-2 gene polymorphisms had a significant influence on the levels of HDL and LDL in the blood of male and female Cobb and Hubbard puppies. (Mean± SE)

IGF-2 genotype	Breed			Sex		
	Cobb	Hubbard	p	Male	Female	p
		high density lipoprotein (IU/Litter)				
TT	84.25	83.73 ±	NS	88.34 ±	88.73 ±	NS
TC	82.65	82.60 ±	NS	87.65 ±	87.92 ±	NS
CC	83.60	82.70 ±	NS	87.60 ±	88.67 ±	NS
p	NS	NS		NS	NS	
		low density lipoprotein (IU/Litter)				
TT	18.55	20.22 ±	0.05	18.55 ±	20.22 ±	0.05
TC	18.33	18.90 ±	NS	18.53 ±	18.25 ±	NS
CC	18.90	18.93 ±	NS	18.65 ±	20.35 ±	0.05
p	NS	0.05		NS	0.05	

"p≤0.05" indicates statistical significance, whereas "NS" indicates no significant difference between genotypes within each breed and gender, as well as between genotypes within each breed and gender within each genotype. Each of the a-b letters represents a 0.05-level variation in genotypes

Table 3. For both males and females of the Cobb and Hubbard breed at 42 days old, IGF-2 gene polymorphisms had an effect on ALT (alanine transamination), aspartate aminotransferase (AST) activity, and alkaline phosphatase activity. (Mean ± SE)

IGF-2 genotype	Breed			Sex		
	Cobb	Hubbard	p	Male	Female	p
alanine transaminase (IU/Litter)						
TT	7.44 ±	6.82 ± 0.28 [b]	0.05	7.43 ±	7.44 ± 0.22	NS
TC	7.40 ±	7.45 ± 0.26 [a]	NS	7.45 ±	7.41 ± 0.26	NS
CC	7.42 ±	7.40 ± 0.22	NS	7.42 ±	7.43 ± 0.28	NS
p	NS	0.05		NS	NS	
aspartate aminotransferase (IU/Litter)						
TT	153.85	156.75 ±	NS	153.28	156.28 ±	NS
TC	152.63	150.72 ±	NS	152.24	156.22 ±	NS
CC	149.86	155.84 ±	0.05	149.93	149.69 ±	0.05
p	0.05	0.05		0.05	0.05	
Alkaline phosphatase (IU/Litter)						
TT	2822	2866 ±	NS	2826	2823 ±	NS
TC	2827	2834 ±	NS	2835±	2873 ±	NS
CC	2868	2873 ±	NS	2873	2876 ±	NS
	±	218.29		±	218.35	
p	NS	NS		NS	NS	

"p0.05" indicates statistical significance, whereas "NS" indicates no significant difference between genotypes within each breed and gender, as well as between genotypes within each breed and gender within each genotype. Genotypes with a-b letters indicate a significant difference at the 0.05 level

Serum levels of ALT and AST

In the Cobb500 breed, the ALT enzyme activity did not vary substantially between the TC and 6.74 IU genotypes, but in the Hubbard F-15 breed, the ALT enzyme activity was considerably greater in the TC genotype than in the 7.40 IU or L genotypes ($p \leq 0.05$). The ALT enzyme activity of Cobb500 broilers and Hubbard F-15 broilers (7.45 vs 7.40 IU / L) differed significantly ($p \leq 0.05$) within the TT genotype of chicken. There were no significant changes in the activity of the ALT enzyme between men and females among the IGF-2 genotypes tested. The AST activity of Cobb500 broilers with the TT or TC genotypes and the CC genotypes varied (p0.05). This was notable in terms of statistics. To the contrary, Hubbard F-15 broilers of the TC genotype exhibited lower AST enzyme activity than those of the TT and CC genotypes in comparison (153.85 versus 152.63 and 149.93, respectively). Hubbard F-15 and Cobb500 broiler outcomes were substantially ($p \leq 0.05$) more active in terms of AST enzyme activity than Cobb500 broiler results. Cobb500 and Hubbard F-15 broilers of the TT and TC genotypes showed no significant variations in AST enzyme activity. The AST enzyme activity in male and female broilers with the TT or TC genetic make-up varied substantially ($p \leq 0.05$) between 149.63 and 153.28 IU/L in males and 149.69 and 156.28 IU/L in females, respectively. We found that men and women with the same genotype have different levels of AST enzyme activity ($p \leq 0.01$).

Activity of the serum alkaline phosphatase enzyme: According to the data, the IGF-2 polymorphism, breed, or gender had no influence on alkaline phosphatase activity in this study. An increase in bone-specific alkaline phosphatase activity was shown to demonstrate IGF-2's anabolic effects on bone tissue (33). It is possible to increase farm animal productivity while preserving genetic diversity using genotypic selection. Based on the results of this and other studies, IGF-2 seems to be a potential candidate gene for use in the chicken breeding program.

Conclusion

As a result of IGF-2 polymorphism in the Cobb500 breed, blood triglyceride contents were lower than those in the Hubbard F-15 breed. In Hubbard F-15 breed, IGF-2 polymorphism had an impact on serum low density lipoprotein (LDL) and serum ALT enzyme concentrations. Serum and AST enzyme concentrations in the Cobb500 and Hubbard F-15 breeds were shown to be affected by a polymorphism in IGF-2. However, IGF-2 polymorphism affected serum LDL concentrations only in female broilers. It was shown that IGF-2 polymorphism affected both male and female broilers in terms of AST enzyme levels in the bloodstream.

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