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Some biochemical traits in broiler chicken as affected by insulin-like growth factor-1 (IGF-1) gene polymorphisms

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Abstract
This study was conducted during the period 1/9/2014 – 1/2/2015 and aimed to identify the polymorphisms of IGF-1 gene in broiler chicken and their effects on some biochemical traits. A total of 300 one-day old broiler chicks (Cobb500, n=150; Hubbard F-15, n=150) were evaluated in this study. Blood samples were individually collected from all birds for DNA extraction. PCR-RFLP method being used for determination the genotypes of IGF-1 gene which then correlated with biochemical traits studied. Cobb500 broilers with TT genotype had significantly ($p<0.05$) higher serum triglycerides values than those of TC and CC genotypes. Low density lipoprotein (LDL) were significantly ($p<0.05$) higher in Hubbard F-15 broilers with TT genotype than those of TC and CC genotypes. Also, LDL were significantly ($p<0.05$) higher in Hubbard F-15 than in Cobb500 broilers. LDL of broilers with TC genotype were significantly ($p<0.05$) higher in males than females. Alanine transaminase (ALT) enzyme activity values were significantly ($p<0.05$) higher in broilers with TC genotype than those of TT genotype. Cobb500 broilers with CC genotype had significantly ($p<0.05$) lower aspartate transaminase (AST) enzyme activity than those of TT and TC genotypes. Hubbard F-15 broilers with TC genotype had significantly ($p<0.05$) lower AST enzyme activity than those of TT and CC genotypes. It was concluded that the activity of ALT and AST enzymes were more influenced by IGF-1 gene polymorphisms.

Keywords: broiler, IGF-1 gene, polymorphism, PCR-RFLP, biochemical

1. Introduction
Chicken insulin-like growth factor I (IGF-1) has been identified as a biological candidate gene responsible for body composition, growth, fat deposition and metabolic activities in chickens [1] and it is a critical regulator of satellite cell proliferation and skeletal muscle hypertrophy [2]. The IGF-1 is produced in the liver in response to the action of the growth hormone in the pituitary [3]. Although the liver is the primary organ where they produce IGF-1, but some organs such as the pituitary, brain, ovary, spleen, and muscle, are also known to synthesize IGF-1 [4]. By binding to the GHR, GH induces the JAK-STAT pathway activation and then triggers IGF-1 synthesis [5]. There are multiple signaling pathways that are involved in IGF-1 inducing muscle cell hyperplasia and hypertrophy, such as PI3K-Akt pathway, mTOR pathway and MAPK pathway [6, 7]. Insulin-like growth factor-1 (IGF-1) is a member of the polypeptide hormone family, preproinsulin, which consisted of proinsulin, IGF-I, IGF-II and C peptide with several metabolic functions [8]. IGF-1 has a great importance during postnatal growth and is mainly produced by the liver under the influence of growth hormone and nutritional conditions and acting in an endocrinological manner on its target tissues [8]. IGFI is located on chromosome 1 within a linkage region where some QTLs controlling growth have been detected [9, 10]. IGF-I is known as one of the more predominant hormones necessary to support normal growth in chickens [11, 12]. Several studies have shown that SNP within the 5' flanking region of IGFI (the promoter region), is significantly associated with the performance of laying hens and broilers. Gouda and Essawy analyzed the polymorphism of IGF-I gene among Egyptian chicken breeds and indicated their effect on the growth traits of chicken was significant [8]. Tang *et al*. [13] studied the association between IGFI gene polymorphism and body weight, age of sexual maturity, egg weight and number in two Chinese (Beijing and Silkies) populations. Al-Hassani *et al*. [14] investigated the relationship between IGF-1 gene polymorphism and body weights in Cobb500 and Hubbard F-15 broiler and they found that IGF-1 gene could be a candidate gene that affects growth in broiler chickens. Kanacki *et al*. [15] indicated that IGF-1 plays an important role in the metabolism of carbohydrates, fats and

Protein in adipose tissue, skeletal muscle and liver. IGF-1 has been suggested to have beneficial effects on glucose homeostasis due to its glucose lowering and insulin sensitizing actions [16]. Clemmons [17] found that IGF-1 can lower blood glucose via inhibition renal gluconeogenesis. The candidate gene approach has become a powerful technique for genetic improvement in the chicken breeding program. Applying a candidate gene may result in higher efficiency in detecting the desired traits necessary to improve production performance. The chicken insulin-like growth factor-I (IGF-I) gene is among the most promising candidate genes for growth performance and carcass quality traits in chickens. The structure of the IGF-1 gene is variable among chicken breeds, but the association of this variability with the phenotypic variation is not yet clear [18]. Therefore, in chicken, the association between variants at the IGF-1 locus and performance traits is required further investigation. The aim of the present study was to identify the polymorphisms of IGF-1 gene at 5'UTR region and to investigate the correlation between these polymorphisms and the phenotype of some biochemical traits in Hubbard F-15 and Cobb500 broiler lines.

2. Material and Methods

2.1 Location and birds

This study was conducted at the poultry farm in animal production department, college of agriculture, the University of Baghdad during a period from 21 September to 25 October 2013, thereafter, in the laboratories of the genetic engineering and biotechnology institute, the University of Baghdad. Cobb500 (n=155) and Hubbard F-15 (n=146) broiler breeds were used in this study. Broiler chicks were obtained from a commercial hatchery and were individually wing banded. The broilers were housed from day 1 to day 49 in experimental house. All housing, feeding and management conditions were in accordance with manual guide of both breeds.

2.2 Sampling

Blood samples were collected at 49 days of age from wing vein then divided into two parts: part one was collected in tubes without anticoagulant to obtain serum and the second part was collected in EDTA-coated tubes for DNA extraction. DNA was extracted using genomic DNA purification kit (Delta Bio Techs, Iraq). The concentration of DNA samples were estimated by Nanodrop using 1µl of the extracted DNA to detect concentration in ng/µL and the purity was detected by ratio of optical density (OD) 260 / 280 nm to detect the contamination of samples with protein. The accepted 260 / 280 ratio for DNA was between 1.7-1.9 [19]. In blood serum the following biochemical compounds being determined; glucose [20], Cholesterol, HDL cholesterol, ALT, AST, ALP [21], triglycerids [22] and LDL cholesterol [21, 23].

2.3 PCR-RFLP assay

The polymorphisms of IGF-1 gene were determined by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The PCR primers for the chicken IGF-1 gene were used (Forward: 5-GACTATACAGAAAGAACCAC-3′; Reverse: 5-TATCACTCAAGTG GCTCAAGT-3′) [24]. PCR reaction was in a total volume of 20µl, containing 3µl of genomic DNA, 10 µM of each oligonucleotide primer, 15 µl of distilled water and PCR premix (Taq DNA polymerase, dNTPs, MgCl2 and reaction buffer (pH= 8.5). Cycle parameters were 94ºC for 5 min. then 35 cycles of 94 ºC for 45 sec., 60 ºC for 45 sec., and 72ºC for 1 min., with a final extension step for 10 min. at 72ºC. PCR products with length 622 bp were digested at 37ºC overnight with 10 U of PstI restriction enzyme. Restriction digests were electrophoresed at 5 volt / cm2 for one hour on a 2% agarose gel with ethidium bromide, and individual PCR-RFLP fragment sizes in each sample were determined based on a standard DNA molecular weight marker by viewing the banding pattern under UV light on the transiluminator. All the three genotypes (TT, TC and CC) were found. The PCR-RFLP analysis using PstI restriction enzyme showed fragments 622 bp for TT genotype; 280, 342 and 622 bp for TC genotype and 280 and 342 bp for CC genotype.

2.4 Statistical analysis

Results were processed and analyzed using GLM procedure in SAS and means were compared using Duncan's new multiple range test [25].

3. Results and Discussion

3.1 Serum glucose concentrations

Based on this study, the results showed that serum glucose concentrations were not affected by IGF-1 genotypes. There were no significant differences in serum glucose concentrations among genotypes within each breed and sex. Moreover, within each genotype, no significant differences in serum glucose concentrations were noted between breeds and between sexes (Table 1). Circulating concentrations of glucose were reported in chickens as 234 mg/dl [26], while, Al-Hassani and Abdul-Hassan found that the plasma glucose of broiler chicken reared under thermoneutral temperature was 212 mg/dl [1]. McMurtry *et al*. [27] speculated that the IGF-1 is involved in establishing a blood glucose baseline. Clemmons [17] observed that IGF-1 can directly lower blood glucose by inhibition renal gluconeogenesis and that IGF-1 can act indirectly, through the IGF-1 receptor in skeletal muscle to enhance insulin action on glucose transport. IGF-1 has been suggested to have beneficial effect on glucose homeostasis due to its glucose lowering and insulin sensitizing action [16].

3.2 Serum cholesterol and triglyceride

As shown in the present study, serum cholesterol concentrations were unaffected by IGF-1 gene polymorphism, breed and sex (Table 1). These results are not in agreement with results obtained by Girbau *et al*. [28] who observed that the injection of IGF-1(10-100 ng/embryo-1) into 2-day-old chicken embryo increased cholesterol of the 4-day age embryo. While, Carolyn *et al*. [29] found an inverse association of IGF-1 with total cholesterol concentrations in human.

Cobb500 broilers with TT genotype had significantly ($p<0.05$) higher serum triglycerides values than those of TC and CC genotypes (92.58 versus 72.02 and 77.37 mg/ dl, respectively). No significant differences were noted in serum triglycerides values among genotypes within males of Hubbard breed and within both sexes, also, between breeds and sexes within each genotype (Table 1). The decline in serum triglyceride values was associated with C allele in Cobb500 breed in this study. It should be pointed out that the levels of cholesterol and triglycerides in serum are genetically-dependent. This may be one of the reasons for their great variability revealed by different researchers in experiments on growing chicken [30].

Table 1: IGF-1 gene polymorphisms effects on glucose, cholesterol and triglycerides concentrations in serum of both sexes for Cobb and Hubbard breeds at 49 days of age. (Mean ± SE)

IGF-1 genotype	Breed			Sex		
	Cobb	Hubbard	*p*	Male	Female	*p*
glucose (mg./100ml)						
TT	144.13 ± 7.29	157.63 ± 11.20	*NS*	152.62 ± 9.15	152.51 ± 13.43	*NS*
TC	151.95 ± 10.71	160.22 ± 5.56	*NS*	154.21 ± 8.41	160.63 ± 5.53	*NS*
CC	163.00 ± 7.59	148.67 ± 6.16	*NS*	155.65 ± 7.32	157.06 ± 6.94	*NS*
p	*NS*	*NS*		*NS*	*NS*	
cholesterol (mg./100ml)						
TT	116.05 ± 5.59	122.90 ± 14.00	*NS*	115.46 ± 11.58	125.20 ± 15.17	*NS*
TC	118.81 ± 5.44	123.59 ± 4.03	*NS*	125.36 ± 4.08	116.27 ± 4.93	*NS*
CC	114.09 ± 3.16	124.56 ± 5.38	*NS*	119.25 ± 3.52	118.33 ± 5.18	*NS*
p	*NS*	*NS*		*NS*	*NS*	
triglyceride (mg./100ml)						
TT	92.58 ± 16.83 [a]	59.91 ± 10.63	*NS*	74.80 ± 19.65	69.52 ± 10.29	*NS*
TC	72.02 ± 0.28 [b]	72.79 ± 4.96	*NS*	77.73 ± 8.19	64.89 ± 3.08	*NS*
CC	77.37 ± 4.26 [b]	70.65 ± 4.84	*NS*	76.43 ± 4.89	71.90 ± 4.13	*NS*
p	*0.05*	*NS*		*NS*	*NS*	

IGF-1 = insulin-like growth factor-1; SE= standard error; p= probability; 0.05= significant at p≤0.05, NS= no significant among genotypes within each breed and sex, also, between breeds and sexes within each genotype. a-b letters indicate a significant difference among genotypes at 0.05 level.

Table 2: The effect of IGF-1 gene polymorphisms on high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations in serum of both sexes for Cobb and Hubbard breeds at 49 days of age. (Mean ± SE)

IGF-1 genotype	Breed			Sex		
	Cobb	Hubbard	*p*	Male	Female	*p*
high density lipoprotein (IU/Litter)						
TT	94.21 ± 5.91	83.73 ± 10.91	*NS*	87.60 ± 10.4	87.73 ± 11.18	*NS*
TC	81.66 ± 3.65	92.65 ± 4.00	*NS*	87.00 ± 4.07	89.82 ± 4.51	*NS*
CC	81.30 ± 2.88	86.66 ± 3.53	*NS*	87.37 ± 3.37	79.97 ± 2.86	*NS*
p	*NS*	*NS*		*NS*	*NS*	
low density lipoprotein (IU/Litter)						
TT	18.66 ± 1.76	27.20 ± 3.80 [a]	*0.05*	24.50 ± 2.72	23.50 ± 5.42 [a]	*NS*
TC	18.00 ± 2.65	17.92 ± 2.90 [b]	*NS*	20.53 ± 2.70	14.22 ± 2.54 [b]	*0.05*
CC	19.93 ± 1.83	18.03 ± 1.48 [b]	*NS*	18.06 ± 1.81	20.10 ± 1.55 [a]	*NS*
p	*NS*	*0.05*		*NS*	*0.05*	

IGF-1 =insulin-like growth factor-1; SE= standard error; p= probability; 0.05= significant at p≤0.05, NS= no significant among genotypes within each breed and sex, also, between breeds and sexes within each genotype. a-b letters indicate, a significant difference among genotypes at 0.05 level.

Table 3: Effect of IGF-1 gene polymorphisms on alanine transaminase (ALT), aspartate aminotransferase AST) and alkaline phosphatase activity in serum of both sexes of Cobb and Hubbard breeds at 49 days of age. (Mean ± SE)

IGF-1 genotype	Breed			Sex		
	Cobb	Hubbard	*p*	Male	Female	*p*
alanine transaminase (IU/Litter)						
TT	7.54 ± 0.19	6.74 ± 0.27 [b]	*0.05*	7.03 ± 0.25	7.04 ± 0.42	*NS*
TC	7.26 ± 0.28	7.31 ± 0.10 [a]	*NS*	7.13 ± 0.20	7.51 ± 0.08	*NS*
CC	7.49 ± 0.14	6.99 ± 0.09 [ab]	*NS*	7.19 ± 0.16	7.03 ± 0.09	*NS*
p	*NS*	*0.05*		*NS*	*NS*	
aspartate aminotransferase (IU/Litter)						
TT	167.05 ± 12.40 [a]	158.86 ± 23.49 [a]	*NS*	150.08 ± 27.63 [a]	173.78 ± 12.25 [a]	*NS*
TC	198.03 ± 16.22 [a]	103.42 ± 19.30 [b]	*NS*	143.77 ± 21.79 [a]	184.19 ± 27.22 [a]	*NS*
CC	102.30 ± 10.31 [b]	144.93 ± 14.39 [a]	*0.05*	117.53 ± 12.13 [b]	127.09 ± 67.12 [b]	*0.05*
p	*0.05*	*0.05*		*0.05*	*0.05*	
Alkaline phosphatase (IU/Litter)						
TT	2149 ± 216.64	2686 ± 267.97	*NS*	2116 ± 423.49	1853 ± 911.94	*NS*
TC	2822 ± 207.40	1358 ± 188.16	*NS*	2334 ± 548.00	1413 ± 521.51	*NS*
CC	2564 ± 318.75	1401 ± 246.19	*NS*	1908 ± 301.69	2146 ± 395.34	*NS*
p	*NS*	*NS*		*NS*	*NS*	

IGF-1 =insulin-like growth factor-1; SE= standard error; p= probability; 0.05= significant at p≤0.05, NS= no significant among genotypes within each breed and sex, also, between breeds and sexes within each genotype. a-b letters indicate a significant difference among genotypes at 0.05 level

3.3 Serum HDL and LDL concentrations

Serum HDL concentrations were not affected by IGF-1 genotypes. Serum HDL concentration were unaffected by IGF-1 gene polymorphism, breed and sex. HDL constituted 38-42% of total cholesterol 32. High density lipoprotein is the main fraction of total cholesterol in the blood of the birds 33. Peebles *et al*. [31] detected the tendency of HDL cholesterol control to decrease with age in meat type chicken 33. There

were no significant differences in serum LDL concentrations among genotypes in Cobb500 breed, while, serum LDL concentrations were significantly ($p<0.05$) higher in Hubbard F-15 broilers with TT genotype than those of TC and CC genotypes (27.20 versus 17.92 and 18.03 IU /L, respectively). Serum LDL concentrations were significantly ($p<0.05$) higher in Hubbard F-15 than in Cobb500 broilers (27.20 versus 18.66 IU / L, respectively). Within TC and CC genotypes, there were no significant differences between Cobb500 and Hubbard F-15 breeds as related with serum LDL concentrations. Within male broilers, no significant differences in LDL concentrations among genotypes were noted, while, within female broilers, LDL concentrations were significantly ($p<0.05$) lower in TC genotype than those of TT and CC genotypes (14.22 versus 23.50 and 20.10 IU / L, respectively). Serum LDL concentrations of broilers with TC genotype were significantly ($p<0.05$) higher in males than females (20.53 versus 14.22 IU / L, respectively). No significant differences in serum LDL concentrations were noted between males and females within TT and CC genotypes. In Hubbard F-15 breed and female broiler in this study, the decrease in serum LDL values was associated with C allele. Hassan *et al.* [32] in Anka and Rugao breeds, found that males compared to females had significantly ($p<0.01$) higher level of LDL in both breeds. These results agree with the present study as related with broiler chickens of TC genotype.

3.4 Serum ALT and AST activity

In Cobb500 breed, there were no significant differences among genotypes as related with ALT enzyme activity, while in Hubbard F-15 breed, ALT enzyme activity values were significantly ($p<0.05$) higher in broilers with TC genotype than those of TT genotype (7.31 versus 6.74 IU / L, respectively). Also, within TT genotype, ALT enzyme activity values were significantly ($p<0.05$) higher in Cobb500 broilers than those of Hubbard F-15 broilers (7.54 versus 6.74 IU / L, respectively). ALT enzyme activity values were unaffected by IGF-1 genotypes in males and females and within each genotype there were no significant differences between males and females as related with ALT enzyme activity values. The AST enzyme activities were higher ($p<0.05$) in Cobb500 broilers with TT and TC genotypes when compared with those of CC genotype (167.05 and 198.03 versus 102.3 IU/L, respectively). In contrast, the AST enzyme activities in Hubbard F-15 broilers were lower ($p<0.05$) in TC genotype when compared with TT and CC genotypes (103.42 versus 158.86 and 144.93, respectively). Within CC genotype, AST enzyme activity values were significantly ($p<0.05$) higher in Hubbard F-15 than in Cobb500 broilers (144.93 versus 102.30 IU / L, respectively). Within TT and TC genotypes, there were no significant differences between Cobb500 and Hubbard F-15 broilers as related with AST enzyme activity values. Male and female broilers with TT and TC genotypes had significantly ($p<0.05$) higher AST enzyme activity than those of CC genotype (150.08 and 143.77 versus 117.53 IU / L, in male broilers; 173.78 and 184.19 versus 127.09 IU / L, in female broilers, respectively). Within CC genotype, AST enzyme activity values were significantly ($p<0.05$) higher in females than males (127.09 versus 117.53 IU / L, respectively).

3.5 Serum alkaline phosphatase activity

The results indicated that alkaline phosphatase activity values were unaffected by IGF-1 polymorphism, breed and sex in this study. It was shown that IGF-1 increases bone –specific alkaline phosphatase activity and oseocation production, proving its anabolic effect on bone tissue [33]. Selection according to genotype has the power to increase productivity of farm animals as well as to enhance environmental adaptation and maintenance of genetic diversity [34]. It appears from the results of the present study and as reported by several authors in previous studies, IGF-1 may consider one of the most promising candidate genes to use in the chicken breeding program.

4. Conclusion

In Cobb500 breed rather than Hubbard F-15 breed, serum triglycerides concentrations were affected by IGF-1polymorphism. Also, in Hubbard F-15 breed rather than Cobb500 breed, serum low density lipoprotein (LDL) and serum ALT enzyme concentrations were affected by IGF-1 polymorphism. While, in both Cobb500 and Hubbard F-15 breeds, serum. AST enzyme concentrations were affected by IGF-1 gene polymorphism. In female rather than male broilers, serum LDL concentrations were affected by IGF-1 polymorphism. In both male and female broilers, serum AST enzyme concentrations were affected by IGF-1 polymorphism.

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