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Association of Insulin-Like Growth Factor-1 Gene Polymorphism at 279 Position of the 5'UTR Region with Body Weight Traits in Broiler Chicken

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ABSTRACT

Insulin-like Growth Factor 1 (IGF-1) gene has been described in several researches as a candidate gene for growth. The present study attempts to identify associations between body weight traits and polymorphism at 279 position of 5'UTR flanking region of IGF-1 gene in broiler chicken. Three hundred broiler chickens from two breeds (Cobb500 and Hubbard F-15) were used in this study. A Single Nucleotide Polymorphism (SNP) at 279 position of 5'UTR region of IGF-1 gene was identified in 20.6 and 60.3% of Cobb500 and Hubbard F-15, respectively, using PCR-RFLP technique. Allele frequencies were 83.87 and 42.80% for T allele and 16.13 and 57.20% for C allele in Cobb500 and Hubbard -15 breeds, respectively. Genotype frequencies were 79.35, 9.03 and 11.61% in Cobb500 and 39.73, 6.16 and 54.11% in Hubbard F-15 for TT, TC and CC genotypes, respectively. As related with mean body weight, different weekly results were noted between Cobb500 and Hubbard F-15 according to genotypes during a period from 3-7 weeks of age. Within all genotypes, the mean body weights were higher ($p>0.05$) in males than females at 5 and 7 weeks of age. As related with body weight gain, Hubbard F-15 broilers were more affected by IGF-1 genotypes than Cobb500 broilers in the last three weeks of age, also, male broilers were more affected by IGF-1 genotypes than female broilers. The results of this study demonstrated that IGF-1 gene, to some extent, could be a candidate gene that affects growth in broiler chickens.

Key words: IGF-1, PCR-RFLP, broiler, body weight

INTRODUCTION

To overcome the negative consequences resulting from genetic selection methods in poultry industry such as obesity, sudden death syndrome and leg problems (Kadlec *et al.*, 2011), alternative efficient methods to be required. The use of molecular marker-assisted selection has proven to be efficient and lead to the improvement in production performance in animals (Fang *et al.*, 2008). Detection of molecular markers for selection of superior poultry stock for economically important traits and the incorporation of this information into breeding plans for improving native poultry performance offers means to incorporate genetic superiority for higher gains for egg and meat productivity (Ali *et al.*, 2013). Niknafs *et al.* (2014) indicated that the integrated of new technologies with traditional methods and identification of effective genes have provided possibilities of more balanced selection.

The ability of meat production is closely associated with muscle growth. Recent researches on polypeptides growth factors have identified several growth factors such as IGFs, epidermal growth factor, transforming growth factor and platelet-derived growth factor as modulators of muscle (Florini *et al.*, 1996; Duclos, 1998). The Insulin-like Growth Factor Gene (IGF1) is a candidate gene for growth, body composition and metabolism, skeletal characteristics and growth of adipose tissue and fat deposition in chickens (Zhou *et al.*, 2005) and Siddiqui *et al.* (1992) reported a positive correlation between IGF-1 concentration and body weight. Nagaraja *et al.* (2000) studied the association between feed intake, body weight, laying rate and egg weight with IGF-1 polymorphism and reported that it was possible to improve the economic traits by IGF-1 genotypes in an unselected population. Amills *et al.* (2003) found suggestive associations between IGF1-SNP and average daily gain at 107 days. Zhou *et al.* (2005) found in the F2 generation of hybrids Leghorn × broiler Fayoum × broiler, a significant association between IGF1-SNP and average daily gains. Fang *et al.* (2008) found a significant correlation between IGF1 polymorphism and egg production. Gouda and Essawy (2010) analyzed the polymorphism of IGF-I gene among Egypt chicken breeds and indicated their effect on the growth traits of chicken was significant. So, Single Nucleotide Polymorphism (SNP) which extensively distributed along the chicken genome has gained interest recently. This study was conducted for identification of the IGF-1 polymorphism and for study its possible association with body weight traits every week of age.

MATERIALS AND METHODS

Experimental chickens and rearing: This study was conducted at Poultry Farm, Animal Resources Department, College of Agriculture, University of Baghdad, during the period from 21 September-25 December 2014. Two broiler breeds-Cobb500 (n = 155) and Hubbard F-15 (n = 146) were used in this study. The sex ratio was 1.06: 1 and 0.97: 1, respectively. Birds were individually marked by wing markers and reared on litter of crushed straw. Feed and water were given *ad libitum*. All chicks were fed a starter diet in mash form containing 23.6% crude protein and 3026 kcal ME/kg during first 10 days of age, then grower diet was fed containing 21.6% crude protein and 3146 kcal ME/kg during 11-24 days of age and finally finisher diet was fed containing 20% crude protein and 3197 kcal ME/kg during 25-49 days. These three diets were formulated according to NRC (1994).

During the experiment, the individual weight of chickens was measured after hatching, in the 7th, 14th, 21st, 28th, 35th, 42nd and 49th of days. The experiment was finished on the 49th day of age of the chickens. Blood samples were collected in EDTA-treated tubes from the 7-week chickens before slaughter for identification of the IGF-1 genotypes of 5'UTR region.

PCR-RFLP assay: For DNA extraction, blood samples were collected in EDTA-treated tubes from the 7 week-old birds before slaughter. The PCR primers for the chicken IGF-1 gene were used (Forward: 5-GACTATACAGAAAGAACCAC-3; Reverse: 5-TATCACTCAAGTGGCTCAAGT-3) (Nagaraja *et al.*, 2000). The DNA amplification by PCR of each bird was performed according to the following conditions: the PCR was performed in a total volume of 20 µL, containing 3 µL of genomic DNA, 10 µM of each oligonucleotide primer, 15 µL of D.W. and PCR premix (*Taq* DNA polymerase, dNTPs, MgCl₂ and reaction buffer (pH = 8.5). Cycle parameters were 94°C for 5 min then 35 cycles of 94°C for 45 sec, 60 °C for 45 sec and 72°C for 1 min, with a final extension step for 10 min at 72°C, the PCR products with length 622 bp were digested at 37°C overnight with 10 U of *Pst* 1 restriction enzyme. Restriction digests were electrophoresed at 5 volt cm⁻² for 1 h 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 transilluminator. All the three genotypes (TT, TC and CC) were found. The Pst1 PCR-RFLP analysis showed fragments 622 bp for TT genotype; 280, 342 and 622 bp for TC genotype and 280 and 342 bp for CC genotype.

Statistical analysis: Obtained results was processed and analyzed using GLM procedure in SAS (SAS., 2010) and means were compared using Duncan's new multiple range test.

RESULTS AND DISCUSSION

Genomic DNA: DNA was extracted from the fresh blood samples of Hubbard F-15 and Cobb500 broiler breeds by using Geneaid kit and the procedure was very efficient and showed a sharp band with a good concentration. After DNA extraction, the concentration and purity of DNA was measured using nanodrop and the results showed that the concentration range was between 30-90 $\mu\text{g } \mu\text{L}^{-1}$ and the purity 1.7-1.9.

PCR analysis: Polymerase Chain Reaction (PCR) was used to amplify a fragment (622 bp) in chicken Insulin-like Growth Factor-1 (IGF-1) gene, which include the targeted polymorphism in 5'UTR (Genbank: EF198877.1). Figure 1 demonstrates the 622 bp fragment loaded with 100 bp DNA ladder in agarose gel electrophoresis.

PCR-RFLP analysis: PCR product (622 bp) mentioned above was subject to digestion with *Pst*1 restriction enzyme to distinguish the three polymorphisms of IGF-1 gene at the site 279 (Thymine to Cytosine transition), this site found within the sequence of the restriction enzyme (CTGCAG) which found in the site sequence 276-281. When the sequence CTGTAG in the reference sequence (EF198877.1) became CTGCAG due to the T to C mutation at the site 279 of 5'UTR in IGF-1 gene, then the enzyme will digest the fragment. After digestion with the restriction enzyme, the homozygous normal (TT) appear as 622 bp, the homozygous mutant (CC) appear as 342 and 280 bp and heterozygous mutant (TC) appear as 280, 342 and 622 bp, as shown in Fig. 2.

Positive results of PCR-RFLP analysis (homozygous and heterozygous mutants) were sent for sequencing to confirm the change in the restriction site (276-281) and to find out other mutations in the studied fragment as shown in Fig. 3.

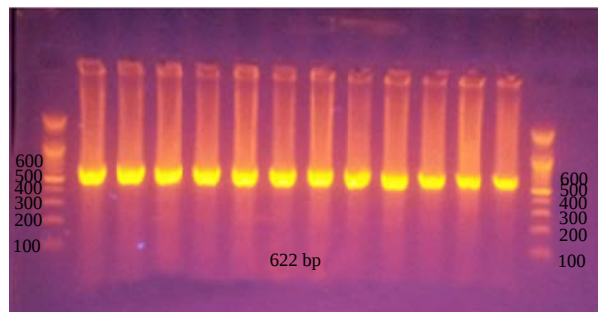


Fig. 1: Genomic DNA was analyzed by electrophoresis on a 1% agarose gel then visualized under U.V. after staining with ethidium bromide

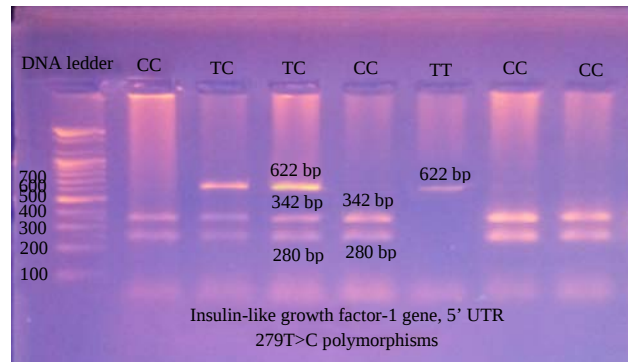


Fig. 2: Representative RFLP analysis of the *Pst*I digest of the PCR product that contains position 279 in 5'UTR of IGF-1 gene separated on a 1% agarose gel, DNA ladder: 100 bp

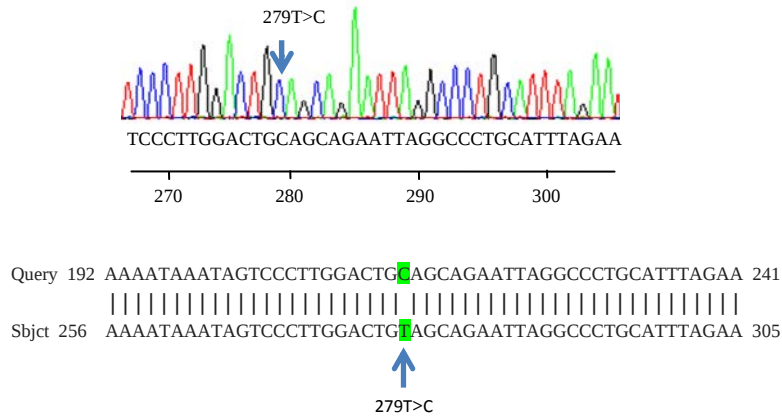


Fig. 3: Electropherogram depicting the 279T>C position and its flanks

Table 1: Genotype and allele frequency, n (%) in Hubbard F-15 and Cobb500 broiler breeds classified according to 279T C SNP in the 5'UTR of IGF-1 gene

	Breed		
Parameters	Cobb500 (No. = 155)	Hubbard F-15 (No. = 146)	p-value
Genotypes			
TT	123 (79.35)	58 (39.73)	0.0019**
TC	14 (9.03)	9 (6.16)	0.427NS
CC	18 (11.61)	79 (54.11)	0.0001**
p-value	0.0026**	0.0139**	
Alleles			
T	260 (83.87)	125 (42.80)	0.00029**
C	50 (16.13)	167 (57.20)	0.00029**
p value	0.0001**	0.0358*	

NS: no significant, *Significant at 0.05, **Significant at and 0.01

Genotype and allele frequency: The genotype and allele frequencies in Hubbard F-15 and Cobb500 broiler chickens that classified according to 279T>C SNP in the 5'UTR of IGF-1 gene are presented in Table 1.

The TT genotype frequency was significantly ($p>0.01$) higher than the frequency of the other genotypes in Cobb500 breed while the CC genotype frequency was significantly ($p>0.01$) higher

than that of the other genotypes in Hubbard F-15 breed. However, TT genotype frequency was significantly ($p>0.01$) higher in Cobb500 breed than in Hubbard F-15 breed (79.35 vs. 39.73%, respectively), whereas, the CC genotype frequency was significantly ($p<0.01$) lower in Cobb500 breed than in Hubbard F-15 breed (11.61 vs. 54.11, respectively). No significant difference was noted between the two breeds as related with TC genotype.

The frequency of T allele was significantly ($p>0.01$) higher than that of C allele in Cobb500 breed (83.87 vs. 16.13%, respectively), in contrast, the frequency of T allele was significantly ($p<0.05$) lower than that of C allele in Hubbard F-15 breed (57.2 vs. 42.8%, respectively). Also, T allele frequency was significantly ($p>0.01$) higher in Cobb500 breed than in Hubbard F-15 breed (83.87 vs. 42.8, respectively), whereas, T allele frequency was significantly ($p<0.01$) lower in Cobb500 breed than in Hubbard F-15 breed (16.13 vs. 57.2, respectively).

The differences in the frequency of genotypes and alleles may be result from the differences in chicken breeds. Li *et al.* (2010) found that the genotypic frequencies of IGF-1 gene in Wenchang fowls were 0.27, 0.41 and 0.32 for genotypes TT, TC and CC and allelic frequencies were 0.47 and 0.53 for alleles T and C, respectively. Khadem *et al.* (2010) found that genotype frequencies in broiler hens of Mazandaran native fowls were 0.18, 0.42 and 0.40 for TT, TC and CC genotypes, respectively, also, the allele frequencies were 0.39 and 0.61 for T and C alleles, respectively. Abbasi and Kazemi (2013) using the *Pst*I enzyme method on IGF1 promoter gene, reported the frequency for C allele was 0.51 while for T allele was 0.49. Furthermore, genotype frequencies for TT, TC and CC were 25.88, 50.23 and 23.89, respectively. Kadlec *et al.* (2011) reported, in Ross 300 strain frequency of C and T alleles in the UTR of IGF-1 gene were 0.915 and 0.085, respectively. In the study of Babayi *et al.* (2014), there was no TT homozygote genotype in west Azerbaijan native poultry. Also, frequency for C allele was 0.83 whereas, for T allele was 0.17 in west Azerbaijan native poultry.

The comparisons of genotype and allele frequencies between males and females of Hubbard F-15 and Cobb500 broiler breeds that classified according to 279T>C SNP in the 5'UTR of IGF-1 gene are shown in Table 2.

As noted in Table 2, the distribution of genotype frequency was in the same trend in males and females and the TT genotype frequency was significantly ($p>0.01$) higher than those of other genotypes in both Cobb500 and Hubbard F-15 breeds (61.94 and 58.68% in male and female, respectively). No significant differences were noted between males and females as related with all genotypes. As related with allele frequency, T allele frequency was significantly ($p>0.01$) higher than the frequency of C allele in both males and females (65.3 vs. 34.7% in males and 62.87 vs. 37.13% in females). No significant difference was noted between males and females as related with T and C alleles.

Body weight: The results of body weights as affected by breed, sex and the genotypes in the 5'UTR of IGF-1 gene in broiler are shown in Table 3.

At one and two weeks of age, the mean body weights were higher ($p>0.05$) in Cobb500 breed than Hubbard F-15 breed within all genotypes. In contrast, the mean body weights were higher ($p>0.05$) in Hubbard F-15 breed than Cobb500 breed within TC and CC genotypes at three weeks of age and there were no significant differences between Cobb500 and Hubbard F-15 breeds within TT genotype. There were no significant differences between the two breeds at four week of age. At five and six weeks of age, the mean body weights were higher ($p>0.05$) in Hubbard F-15 breed than Cobb500 breed within TT genotype, whereas, in TC genotype were higher ($p>0.05$) in Cobb500

Table 2: Genotype and allele frequency, n (%) in Male and Female of Hubbard F-15 and Cobb500 broiler breeds classified according to 279T>C SNP in the 5'UTR of IGF-1 gene

Parameters	Sex		p-value
	Male (No. = 134)	Female (No. = 167)	
Genotypes			
TT	83 (61.94)	98 (58.68)	0.682NS
TC	9 (6.72)	14 (8.38)	0.702NS
CC	42 (31.34)	55 (32.93)	0.763NS
p-value	0.00062**	0.0059**	
Alleles			
T	175 (65.30)	210 (62.87)	0.652NS
C	93 (34.70)	124 (37.13)	0.652NS
P-value	0.0017**	0.0004**	

NS: No significant, **Significant at 0.01

Table 3: Weekly body weights and gains of Cobb-500 and Hubb^ard F-15 broilers as affected by polymorphism in 5'UTR region of IGF-1 gene

Trait (Unit)	Age (days)	Male broilers			Female broilers		
		TT	TC	CC	TT	TC	CC
Body weight (g)	7	67.80±3.8000 ^a	68.80±1.500 ^a	70.90±1.100 ^a	65.70±3.000 ^a	64.70±1.30 ^a	68.90±1.000 ^a
	14	130.00±6.9000 ^a	133.90±3.300 ^a	140.70±2.300 ^a	141.10±6.600 ^a	131.90±2.400 ^a	149.50±9.400 ^a
	21	402.80±19.600 ^a	419.60±9.500 ^a	423.10±5.300 ^a	407.90±9.900 ^a	416.60±5.900 ^a	410.50±4.900 ^a
	28	865.00±39.000 ^a	866.70±24.50 ^a	908.50±12.90 ^a	868.60±17.20 ^a	865.60±10.40 ^a	853.50±9.000 ^a
	35	1431.70±36.500 ^a	1475.20±28.60 ^a	1514.30±15.90 ^a	1349.60±23.20 ^a	1354.10±17.10 ^a	1363.70±12.40 ^a
	42	1891.10±54.700 ^a	1948.70±39.30 ^a	1981.10±40.90 ^a	1762.50±27.90 ^a	1768.00±25.80 ^a	1766.80±22.10 ^a
	49	2763.90±134.20 ^a	2838.30±54.70 ^a	2729.30±37.00 ^a	2381.80±63.80 ^a	2459.10±36.50 ^a	2354.30±24.20 ^a
Body weight gain (g)	1-7	26.44±3.6600 ^a	29.61±1.360 ^a	28.27±0.940 ^a	25.71±2.790 ^a	28.52±1.220 ^a	28.61±0.890 ^a
	8-14	62.22±4.9300 ^a	65.04±2.450 ^a	69.75±1.780 ^a	75.35±5.330 ^a	67.18±1.780 ^a	80.67±0.250 ^a
	15-21	272.77±15.360 ^a	285.78±8.620 ^a	282.40±4.450 ^a	266.78±5.300 ^a	284.72±4.760 ^a	260.94±10.13 ^a
	22-28	462.22±22.480 ^a	447.02±19.18 ^a	485.43±11.48 ^a	460.71±10.28 ^a	448.94±5.490 ^a	443.00±7.730 ^a
	29-35	566.66±28.620 ^a	608.57±20.58 ^a	605.74±16.84 ^a	481.07±18.97 ^a	488.50±12.52 ^a	510.25±9.860 ^a
	36-42	459.44±40.940 ^a	473.45±20.71 ^a	466.79±37.90 ^a	412.85±21.60 ^a	413.90±16.92 ^a	403.08±21.61 ^a
	43-49	872.77±112.42 ^a	889.64±30.71 ^a	748.20±45.83 ^a	619.28±57.82 ^a	691.07±30.32 ^a	587.55±29.72 ^a

Different small letters within each breed at each age refer to a significant difference at (p<0.05), Values are Mean±standard error

breed than Hubbard F-15 breed and there is no difference between Cobb500 and Hubbard F-15 within CC genotype. At seven week of age, the mean body weights of Hubbard F-15 breed were higher (p>0.05) than those of Cobb500 breed within all genotypes.

There were no significant differences in mean body weights among genotypes of IGF-1 gene at all ages except for Cobb500 breed at one week of age, the mean body weights in TC genotype were higher (p>0.05) than those of TT and CC genotypes.

There were no significant differences in mean body weights between males and females within each genotype at one week of age. At 2, 3 and 4 weeks of age, there were no significant differences in mean body weights between males and females within TT and TC genotypes. At 2 week of age, the mean body weights were higher (p>0.05) in females than males within CC genotype while, the mean body weights were higher (p>0.05) in males than females within CC genotype at 3 and 4 weeks of age. At 6 week of age, there were no significant differences in mean body weights between males and females within TT genotype, whereas, the body weights were higher (p>0.05) in males than females within TC and CC genotypes. Within all genotypes, the mean body weights were higher (p>0.05) in males than females at 5 and 7 weeks of age.

In this study, live body weights at 49 days of age were unaffected by IGF-1 genotypes. Also, live body weights of Hubbard F-15 breed were higher than those of Cobb500 breed. Live body

weights of males were higher than those of females. These results are disagree with the results of Zhou *et al.* (2005) who indicated that IGF-I gene polymorphism was associated with growth, body composition, skeleton integrity and metabolic traits in chickens. Also, disagree with the results of Gouda and Essawy (2010) who analyzed the polymorphism of IGF-I gene among Egypt chicken breeds and indicated their effect on the growth traits of Table 3 chicken was significant. The results of this study are agree with the results of Nagaraja *et al.* (2000) who observed that IGF-1 genotype did not give different weight in chickens aged 140, 265 and 365 days. In Lohman chickens, Wang *et al.* (2004) indicated that the highest body weight of broiler chickens were in genotype CC then TC and TT genotypes. Fang *et al.* (2008) found that the IGF-1 gene has a very significant effect on body weight, egg weight in Xinghua chickens. Harini *et al.* (2013) found that the TT genotype at all ages have body weight lower than chicken with genotype TC.

Body weight gain: The results of the effect of different polymorphisms in 5'UTR of IGF-1 gene as affected by breed and sex on weekly body weight gain are presented in Table 4.

During the 1st week of age, there were no significant differences between breeds, sexes and genotypes as related with body weight gain. During the 2nd week of age, body weight gains were higher ($p>0.05$) in Cobb500 breed than Hubbard F-15 breed within all genotypes. Also, within each breed, there were no significant differences in body weight gains among all genotypes. Body weight gains were significantly ($p>0.05$) higher in females than males within TT and CC genotypes while, no significant differences were noted between males and females within TC genotype. The body weight gains for broilers with CC genotype were significantly ($p>0.05$) higher than for broilers with TT and TC genotypes.

During the 3rd week of age, within all genotypes, the body weight gains were in Hubbard F-15 breed significantly ($p>0.05$) higher than in Cobb500 breed and there were no significant differences among genotypes. Within CC genotype, the body weight gains for males were significantly ($p>0.05$) higher than in females. In males with TC and CC genotypes, body weight gains were significantly ($p>0.05$) higher than those with TT genotype. In females, there were no significant differences among all genotypes as related with body weight gains.

During the 4th week of age, there were no significant difference among genotypes within each breed, also, between Cobb500 and Hubbard F-15 breeds within each genotype as related with body weight gain. Body weight gains for males with CC genotype were significantly ($p>0.05$) higher than

Table 4: Weekly body weights and gains of male and female broilers as affected by polymorphism in 5'UTR region of IGF-1 gene

	Age (days)	Cobb-500 genotypes			Hubbard F-15 genotypes		
		TT	TC	CC	TT	TC	CC
Body weight (g)	7	68.20±3.500 ^b	74.40±1.700 ^a	72.300±0.800 ^b	63.90±2.200 ^a	64.70±1.1000 ^a	64.40±1.200 ^a
	14	145.70±6.000 ^a	150.30±5.400 ^a	154.400±7.600 ^a	122.80±5.800 ^a	128.80±1.8000 ^a	126.90±2.300 ^a
	21	405.40±14.00 ^a	404.70±10.50 ^a	411.4000±4.50 ^a	406.70±12.10 ^a	420.90±6.0000 ^a	426.10±6.000 ^a
	28	867.10±27.80 ^a	857.80±32.40 ^a	874.700±10.10 ^a	867.20±17.70 ^a	867.90±12.900 ^a	885.30±11.80 ^a
	35	1347.90±24.10 ^a	1440.30±37.70 ^a	1426.100±13.60 ^a	1434.40±34.00 ^a	1398.90±18.800 ^a	1441.70±20.40 ^a
	42	1774.60±34.80 ^a	1924.20±65.50 ^a	1863.100±31.50 ^a	1872.20±49.40 ^a	1828.50±25.300 ^a	1861.70±29.10 ^a
	49	2364.60±54.80 ^a	2555.00±102.8 ^a	2470.400±28.00 ^a	2790.60±134.3 ^a	2638.90±38.600 ^a	2631.80±49.80 ^a
Body weight gain (g)	1-7	24.85±3.370 ^a	30.94±1.810 ^a	28.510±0.790 ^a	27.77±1.960 ^a	28.55±1.0300 ^a	28.34±1.120 ^a
	8-14	77.50±4.530 ^a	75.83±4.410 ^a	82.048±7.420 ^a	58.88±5.570 ^a	64.07±1.3900 ^a	62.50±5.570 ^a
	15- 21	259.64±9.320 ^a	254.44±8.320 ^a	257.056±8.280 ^a	283.88±6.750 ^a	292.15±4.9900 ^a	299.15±4.510 ^a
	22-28	461.78±15.79 ^a	453.05±29.26 ^a	463.260±9.390 ^a	460.55±11.94 ^a	446.987±8.795 ^a	459.29±7.790 ^a
	29-35	480.71±15.69 ^b	582.50±3492 ^b	551.380±13.36 ^a	567.22±33.08 ^a	530.92±13.520 ^a	556.37±12.64 ^a
	36-42	426.78±28.59 ^a	483.88±36.10 ^a	437.060±28.96 ^a	437.77±30.74 ^a	429.62±14.130 ^a	419.98±21.44 ^a
	43-49	590.00±57.73 ^a	630.83±72.67 ^a	607.280±35.19 ^a	918.30±96.71 ^a	810.36±22.800 ^a	770.06±34.30 ^a

Different small letters within each sex at each age refer to a significant difference at ($p<0.05$). Values are Mean±SE

those with other genotypes. Also, within CC genotype, the body weight gains were significantly ($p>0.05$) higher in males than females. In females, there were no significant differences among genotypes as related with body weight gains.

During the 5th week of age, there was a significant difference in body weight gain among genotypes within each breed. Body weight gains were in males significantly ($p>0.05$) higher than females within TT and TC genotypes. In all genotypes, body weight gains were in males significantly ($p>0.05$) higher than females. There were no significant differences among genotypes within males and females.

During 6th week of age, there were no significant differences among genotypes of Cobb500 breed as related with body weight gain while, Hubbard F-15 broilers with TT and TC genotypes were significantly ($p>0.05$) higher in body weight gains than those with CC genotype. Within CC and TC genotypes, body weight gains were in Cobb500 breed significantly ($p>0.05$) higher than Hubbard F-15 breed while, no significant difference was found between the two breeds within TT genotype. In all Table 4 genotypes, the body weight gains for males were significantly ($p>0.05$) higher than for females.

During the 7th week of age, body weight gains for Hubbard F-15 breed were significantly ($p>0.05$) higher than for Cobb500 breed in all genotypes. In Hubbard F-15 breed, body weight gains were in TT and TC genotypes significantly ($p>0.05$) higher than in CC genotype. No significant differences in body weight gains were noted among genotypes of Cobb500 breed. In all genotypes, body weight gains were significantly ($p>0.05$) higher in males than females. Body weight gains in males with TT and TC genotypes were significantly ($p>0.05$) higher than those with CC genotype and there were no significant differences in body weight gains among genotypes of females.

As related with body weight gain, Hubbard F-15 broilers were more affected by IGF-1 genotypes than Cobb500 broilers in the last three weeks of age, also, male broilers were more affected by IGF-1 genotypes than female broilers.

Associations of an IGF1 promoter polymorphism with Average Daily Gain (ADG) were found in 2 genetically diverse Black Penedesenca chicken strains (A Mills *et al.*, 2003). Amills *et al.* (2003) found suggestive associations ($p\leq 0.05$) between IGF1-SNP and average daily gain at 107 days. Zhou *et al.* (2005) found in the F2 generation of hybrids Leghorn×broiler Fayoum×broiler, a significant association (at 5% significance level) between IGF1-SNP and average daily gains. Some results of research on the IGF-1 gene polymorphism is associated with growth have been reported in chickens (Sco *et al.*, 2001; Kita *et al.*, 2005; Li *et al.*, 2009), in sheep (Zhang *et al.*, 2008) and in cattle (Curi *et al.*, 2006; Siadkowska *et al.*, 2006; Arman *et al.*, 2012).

CONCLUSION

The conclusion of this study demonstrated that IGF-1 gene, to some extent, could be a candidate gene that affects growth in broiler chickens.

REFERENCES

- Abbasi, H.A. and M. Kazemi, 2013. Detection of polymorphism of the insulin-like growth factor-I (IGF-I) gene in Mazandaran native chicken using PCR-RFLP method. *Afr. J. Biotechnol.*, 10: 13351-13354.
- Ali, A., K. Javed, A. Ali, M. Akram, H.M. Pasha, A. Ali and S. Ahmad, 2013. Detection of polymorphism of Insulin-like growth factor-I (IGF-I) gene in native Aseel chicken breed of Pakistan using PCR-RFLP. *Agric. Adv.*, 2: 173-176.

- Amills, M., N. Jimenez, D. Villalba, M. Tor and E. Molina *et al.*, 2003. Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits. *Poult. Sci.*, 82: 1485-1493.
- Arman, C., C. Sumantri and E. Gurnadi, 2012. A novel single nucleotide polymorphism in exon 4 of insulin-like growth factor-1 associated with production traits in bali cattle. *Media Peternakan-J. Anim. Sci. Technol.*, 35: 96-101.
- Babayi, M.S., H. Seyedabadi, A. Gorbani and N. Zarghami, 2014. IGF-I gene polymorphism and its associations with some growth traits in west-Azerbaijan native chicken using PCR-RFLP Techniques. *Bull. Env. Pharmacol. Life Sci.*, 3: 29-32.
- Curi, R.A., H.N. de Oliveira, A.C. Silveira and C.R. Lopes, 2006. Association between IGFI,IGF-IR and GHRH gene polymorphisms and growth and carcass traits in beef cattle. *Livestock Prod. Sci.*, 94: 159-167.
- Duclos, M.J., 1998. Regulation of chicken muscle growth by insulin like growth Factorsa. *Ann. N. Y. Acad. Sci.*, 839: 166-171.
- Fang, L.H., W. Zhu, K. Chen, X. Wu, Q. Tang and Y. Gao, 2008. Associations between GHR and IGF-1 gene polymorphisms and reproductive traits in wenchang chickens. *Turk. J. Vet. Anim. Sci.*, 32: 281-285.
- Florini, J.R., D.Z. Ewton and S.A. Coolican, 1996. Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr. Rev.*, 17: 481-517.
- Gouda, E.M. and G.S. Essawy, 2010. Polymorphism of insulin-like growth factor I gene among chicken breeds in Egypt. *Zeitschrift fur Naturforschung*, 65: 284-288.
- Harini, N.M., S. Duryadi, S. Sri and S. Cece, 2013. Polymorphisms of Insuline-like growth factor-I (IGF-1) and pituitary positive transcription factor-1 (Pit-I) genes and their effect on growth traits in indonesian native chickens. *Proceeding of the International Conference on 4th Green Technology Faculty of Science and Technology*, January 2013, State Islamic University of Malang, Malang.
- Kadlec, J., B. Hosnedlova, V. Rehout, J. Citek, L. Vecerek and L. Hanusova, 2011. Insulin-like growth factor-I gene polymorphism and its association with growth and slaughter characteristics in broiler chickens. *J. Agrobiol.*, 28: 157-163.
- Khadem, A., H. Hafezian and G. Rahimi-Mianji, 2010. Association of single nucleotide polymorphisms in IGFI, IGF-II and IGFBP-II with production traits in breeder hens of Mazandaran native fowls breeding station. *Afr. J. Biotechnol.*, 9: 805-810.
- Kita, K., K. Nagao and J. Okumura, 2005. Nutritional and tissue specificity of IGF-I and IGFBP-2 gene expression in growing chickens*-A review. *Asian-Aust. J. Anim. Sci.*, 18: 747-754.
- Li, H., W. Zhu, K. Chen, W. Song, J. Shu and W. Han, 2010. Effect of the polymorphism of GHR gene and IGF-I gene on egg quality in wanchang chicken. *J. Medwell*, 3: 19-22.
- Li, H.F., W. Zhu, K. Chen, X. Wu and Q. Tang *et al.*, 2009. Polymorphism in NPY and IGF-I genes associate with reproductive traits in Wenchang chicken. *Afr. J. Biotechnol.*, 8: 4744-4748.
- NRC., 1994. *Nutrient Requirements of Poultry*. 9th Edn., National Academy Press, Washington, DC.
- Nagaraja, S.C., S.E. Aggrey, J. Yao, D. Zadworny, R.W. Fairfull and U. Kuhnlein, 2000. Brief communication. Trait association of a genetic marker near the IFG-I gene in egg-laying chickens. *J. Heredity*, 91: 150-156.

- Niknafs, S., A.N. Javaremi and M. Sadeghi, 2014. Single nucleotide polymorphisms in BMPR-IB and STAT5B genes and their association with growth and reproductive traits in chicken. *Songklanakarin J. Sci. Technol.*, 36: 137-142.
- SAS., 2010. Statistical Analysis System, Systems for Windows. SAS Institute Inc., Cary.
- Sco, D.S., J.S. Yun, W.J. Kang, G.J. Jeon K.C. Hong and Y. Ko, 2001. Association of insulin-like growth factor-I gene polymorphism with serum IGF-I concentration and body weight in Korea native Ogo Chicken. *Asian-Aust. J. Anim. Sci.*, 14: 915-921.
- Siadkowska, E., L. Zwierzchowski, J. Oprzadek, N. Strzałkowska, E. Agnicka and J. Krzyzewski, 2006. Effect of polymorphism in IGF-1 gene on production traits in Polish Holstein-Friesian cattle. *Anim. Sci. Pap. Rep.*, 3: 225-237.
- Siddiqui, R.A., S.N. McCutcheon, H.T. Blair, D.D. Mackenzie, P.C. Morel, B.H. Breier and P.D. Gluckman, 1992. Growth allometry of organs, muscles and bones in mice from lines divergently selected on the basis of plasma insulin-like growth factor-I. *Growth Dev. Aging.*, 56: 53-60.
- Wang, W., K. Ouyang, J. Ouyang, H. Li, S. Lin and H. Sun, 2004. Polymorphism of insulin-like growth factor I gene in six chicken breeds and its relationship with growth traits. *Asian Aust. J. Anim. Sci.*, 17: 301-304.
- Zhang, C., W. Zhang, H. Luo, W. Yue, M. Gao and Z. Jia, 2008. A new single nucleotide polymorphism in the IGF-I gene and its association with growth traits in the Nanjiang Huang goat. *Asian-Aust. J. Anim. Sci.*, 21: 1073-1079.
- Zhou, H., A.D. Mitchell, J.P. McMurtry, C.M. Ashwell and S.J. Lamont, 2005. Insulin-like growth factor-I gene polymorphism associations with growth, body composition, skeleton integrity and metabolic traits in chickens. *Poult. Sci.*, 84: 212-219.