PCR-RFLP Analysis of Insulin-Like Growth Factor 2 Gene Polymorphisms in Two Commercial Broiler Chicken Strains (Cobb 500 and Hubbard F-15) and Their Associations with Performance Traits

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ABSTACT

In two commercial broiler breeds (Cobb 500 and Hubbard F-15), the polymorphisms of the chicken insulin-like growth factor 2 (*IGF2*) gene were studied. A total of three hundred avian blood samples were obtained. Using a fast salt-extraction technique, genomic DNA was isolated. Using polymerase chain reaction, 1146 bp fragments of the gene were amplified (PCR). The amplified fragments were subjected to restriction enzyme digestion using HinfI endonuclease enzyme, and the digested products were separated on a 2% agarose gel. The findings indicated two alleles T and C for the target locus, with respective frequencies of 73.3% and 26.7%. Three distinct genotype variations, TT, TC, and CC, were found, with genotype frequencies of 59.1 percent, 28.4 percent, and 12.5 percent, respectively. The test based on actual and anticipated frequencies of various genotypic variances of the *IGF2* gene revealed that the divergence from Hardy-Weinberg equilibrium was not significant ($P \leq 0.01$) in commercial broiler breeds (Cobb 500 and Hubbard F-15) chickens. In addition, it is shown that birds with genotype TC had a greater body mass at 8 weeks of age compared to those with genotypes TT and CC. It was determined that the *IGF*2 gene exhibited a significant degree of variability and might be regarded a possible genetic marker in selection and breeding programs for poultry.

KEY WORDS CHICKEN; PCR; POLYMORPHISM; *IGF*2

INTRODUCTION

Insulin like growth factor 2 (*IGF2*) is a mutagenic polypeptide with an insulin-like structure that controls chicken primary growth. The genes for insulin-like growth factor are situated on chromosome 5, which has two intron and three exon regions [1, 2]. The introns and exons of the chicken *IGF2* gene resemble those of the mouse and human *IGF2* genes [1]. Composed of 187 amino acids, comprising 24 signal peptides, 67 *IGF2* peptides, and 96 amino acids for its C-terminal portion, chicken *IGF2* has 33 amino acids in common with its rat counterpart and 82 amino acids with its human counterpart [1]. Numerous studies on various mammalian species have indicated that *IGF-1* has a wide effect on growth [3]. Similar to mammals, the function of the *IGF* system in chickens [3, 4]. Chicken tissues are stimulated to develop and differentiate by insulin-like growth factors. These variables primarily influence the rate of protein synthesis, DNA synthesis, and substrate transition. In addition to altering chicken body and muscle development, insulin-like growth hormones may also impact ovulation rates and ovarian follicle extension [4]. In addition to acting on insulin-like receptors, this gene decreases blood glucose [5]. Compared to embryonic *IGF2* gene transcripts, there are significantly less *IGF2* peptides in chicken embryos, as shown by gene expression [6]. Two of the ten kinds of *IGF1*-binding proteins found in mammals are significantly expressed in chickens. Studies in vitro have shown that

myoblasts and satellite cells in chicken embryos release binding proteins [7]. The chicken *IGF-1*, *IGF-2*, and *IGF-3* are polypeptide hormones that contribute to their function by binding to particular type 1 receptors [8]. There is evidence that *IGF2* gene inheritance is paternal in several placental animals [9, 10]. *IGF2* influences the hens' development rate, body composition, and lipid metabolism [3, 11, 12]. Insulin-like growth factors have a significant influence on the embryonic development and differentiation of several animal species. In addition, research has shown that *IGF* is the primary gene responsible for the overweight of hens [4, 13, 14, 15]. *IGFs* work in accordance with the paracrine system of the body [16]. Comprised of peptide hormones, cell surface receptors, and binding proteins, the *IGF* system is a complicated system. *IGF-1* and *IGF*-2 hormones bind to insulin-like growth factor receptor 1 and insulin receptor, respectively, and activate the intrinsic and main actions of tyrosine kinase [16]. *IGF*2 has also shown to affect the wasting of muscles in rats, pigs, and cows, as well as the lipid metabolism in poultry [17]. *IGF*2 is a molecular marker for the selection of hens with low abdominal fat [13, 14, 15] , since it is the primary gene influencing chicken obesity. In this study, the polymorphism of the promoter region of Insulin-like growth factor in commercial broiler breeds (Cobb 500 and Hubbard F-15) chickens were analyzed using the PCR-RFLP technique, and the allele and genotype frequencies, as well as the association between these polymorphisms and chicken growth traits, were determined.

MATERIALS AND METHODS

A random selection of three hundred chicks was used from the commercial broiler breeds (Cobb 500 and Hubbard F-15) chickens. The breeding facility provided the performance characteristic data, including their weight at 1st to 42 days of age. Blood was extracted from the wing veins of chickens and preserved in EDTA-coated sterile tubes containing 1.5 ml. The blood samples were transported to the college of veterinary medicine's central laboratory. 200 ml of blood were used for genomic DNA extraction. The DNA was extracted using the rapid salt-extraction technique [18]. A primer combination consisting of (*IGF*2-F) 5'-CCA GTG GGA CGA AAT AAC AGG AGG A-3' and (*IGF*2-R) 5'-TTC CTG GGG GCC GGT CGC TTC A-3' was used to amplify 1146 bp fragment of the *IGF*2 gene [18]. PCR was performed in 25 l reaction volume comprising 50 mM of dATP, dTTP, dCTP, and dGTP, 0.5 mM of each primer, 2.5 l of 10X PCR buffer , 2 mM magnesium chloride, 2.5 U of Taq DNA polymerase , and 50 ng of extracted DNA as template. 35 cycles of denaturation at 94°C for 1 minute, annealing at 67°C for 3 minutes, elongation at 72°C for 3 minutes, and final extension at 72°C for 5 minutes comprised the amplification procedure. The PCR products were separated on an agarose gel containing 1.5 percent agarose, and the gel was imaged using UV trans-illumination. With HinfI restriction endonuclease, PCR products were
digested. In 15 l mixtures comprising 5 l of PCR product, 5U Hinf I endonuclease, and 1.5 l Hinf I buffer, the digestion process was carried out. The mixes were incubated for two hours at 37 °C. The fragments of digested DNA were then run on a 1.5 percent agarose gel and imaged using UV trans-illumination. Using PopGene32 version 1.23 [19], the allelic and genotypic frequencies and observed and anticipated heterozygosity's were calculated. The Hardy-Weinberg equilibrium test was also conducted using PopGene 32. The percentages of homozygosity and heterozygosity were determined.

Results and Discussion

Using the given primers, PCR products of size 1146 bp were produced satisfactorily. All extracted genomic DNA from chicken blood samples generated a PCR result with a single, specific band and no nonspecific bands. Consequently, the PCR results were used immediately for RFLP analysis. Figure

1 depicts RFLP patterns generated by HinfI digestion of PCR products derived from the *IGF*2 gene. Two alleles, T and C, with respective frequencies of 73.3 and 26.7 percent, and three genotypes, TT, TC, and CC, with respective frequencies of 59.1 percent, 28.4 percent, and 12.5 percent, were found (Table 1). The TT homozygous genotype was discovered to be the most common genotype.

Table 1 displays the genetic variability of *IGF*-2 exons 2 and 3 in commercial broiler breeds (Cobb 500 and Hubbard F-15).

Genetic diversity statistics	**Value**	**Allele frequencies**	**Value**	**Genotype Frequencies**	**Value**
NA	2	T	0.733	TT	0.591
NE	1.4	C	0.260	TC	0.284
Observed homozygosity	0.7159	-	-	CC	0.125
Observed heterozygosity	0.2841	-	-	-	-
Expected homozygosity	0.6063	-	-	-	-
Expected heterozygosity	0.3937	-	-	-	-
Average heterozygosity	0.3915	-	-	-	-
Nei Heterozygosity	0.3915	-	-	-	-

NA = observed number of alleles, NE = effective number of alleles

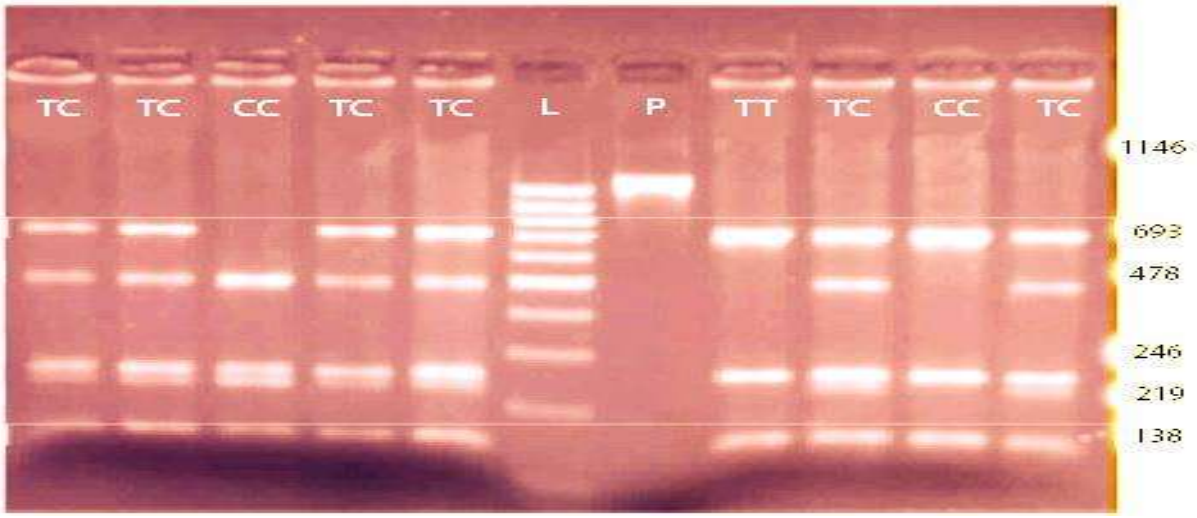

Figure 1 depicts RFLP patterns generated by Hinf1 enzyme cutting and electrophoresed on 2% agarose gel. L the molecular marker for German Ferments (50 bp or more), There are three types of genotype, TT, TC and CC genotypes

Table 3. The effect of different polymorphisms in 5'UTR of IGF-2 gene on weekly body weight (gram) in both sexes of Cobb and Hubbard broiler breeds. (Mean ± SE).

Age (weeks)	Factors		Genotype			*p*
			TT	**TC**	**CC**	
1	**Breed**	**Cobb**	67.0±2.4 **b**	75.2±1.7 **a**	67.3±1.8 **b**	***0.05***
		Hubbard	68.2±2.2 **a**	65.6±1.0 **a**	65.3±1.3 **a**	***NS***
	p		*0.05*	*0.05*	*0.05*	
	Sex	**Male**	68.2±2.8 **a**	70.2±1.6 **a**	68.2±1.3 **a**	***NS***
		Female	67.2±2.0 **a**	64.7±1.3 **a**	68.2±1.2 **a**	***NS***
	p		***NS***	***NS***	***NS***	
2	**Breed**	**Cobb**	153.3±6.2 **a**	152.0±5.4 **a**	153.4±5.6 **a**	***NS***
		Hubbard	128.2±5.8 **b**	126.3±5.2 **b**	127.0±5.3 **a**	***NS***
	p		***0.05***	***0.05***	***0.05***	
	Sex	**Male**	152.0±6.5 **a**	153.6±5.3 **a**	152.6±5.3 **a**	***NS***
		Female	128.2±6.6 **b**	126.4±5.4 **b**	127.5±5.4 **b**	***NS***
	p		***0.05***	***0.05***	***0.05***	
3	**Breed**	**Cobb**	406.4±12.0 **b**	406.3±12.5 **b**	407.6±12.5 **b**	***NS***
		Hubbard	418.7±12.2 **a**	420.0±12.0 **a**	417.5±12.0 **a**	***NS***
	p		***0.05***	*0.05*	*0.05*	
	Sex	**Male**	416.4±12.6 **a**	420.6±12.5 **a**	416.3±12.3 **a**	***NS***
		Female	406.9±12.0 **b**	408.6±12.9 **b**	407.0±12.6 **a**	***NS***
	p		***0.05***	***0.05***	*0.05*	

4	**Breed**	**Cobb**	856.2 ± 12.8 **b**	857.8 ± 12.6 **b**	854.6 ± 12.3 **b**	***NS***
		Hubbard	876.3 ± 12.7 **a**	877.3 ± 12.4**a**	875.3 ± 12.0 **a**	***NS***
	p		***0.05***	***0.05***	***0.05***	
	Sex	**Male**	876.0 ± 12.0 **a**	878.7 ± 12.5 **a**	876.5 ± 12.5 **a**	***NS***
		Female	856.2 ± 12.2 **b**	858.6 ± 12.4 **b**	855.5 ± 12.0 **b**	***NS***
	p		***0.05***	***0.05***	*0.05*	
5	**Breed**	**Cobb**	1343.2 ± 20.0 **b**	1342.2 ± 20.3 **b**	1340.0 ± 20.6 **b**	***NS***
		Hubbard	1435.0 ± 20.3 **a**	1438.9 ± 20.2 **a**	1436.6 ± 20.4 **a**	***NS***
	p		*0.05*	*0.05*	***0.05***	
	Sex	**Male**	1435.67 ± 20.5 **a**	1440.3 ± 20.6 **a**	1438.2 ± 20.6 **a**	***NS***
		Female	1348.6 ± 20.2 **b**	1345.2 ± 20.3 **b**	1343.7 ± 20.3 **b**	***NS***
	p		*0.05*	*0.05*	*0.05*	
6	**Breed**	**Cobb**	1775.3 ± 25.6 **b**	1774.2 ± 25.5 **b**	1773.2 ± 25.5 **b**	***NS***
		Hubbard	1822.2 ± 25.4 **a**	1828.5 ± 25.3 **a**	1826.6 ± 25.3 **a**	***NS***
	p		*0.05*	*0.05*	***0.05***	
	Sex	**Male**	1822.1 ± 25.6 **a**	1828.7 ± 25.3 **a**	1826.3 ± 25.6 **a**	***NS***
		Female	1772.5 ± 25.5 **b**	1774.0 ± 25.0 **b**	1776.8 ± 25.2 **b**	***NS***
	p		***0.05***	*0.05*	*0.05*	
7	**Breed**	**Cobb**	2365.8 ± 38.2 **b**	2366.0 ± 38.2 **b**	2363.4 ± 38.0 **b**	***NS***
		Hubbard	2763.2 ± 38.0 **a**	2768.9 ± 38.6 **a**	2761.8 ± 38.3 **a**	***NS***
	p		*0.05*	*0.05*	*0.05*	
	Sex	**Male**	2763.2 ± 38.2 **a**	2768.2 ± 38.7 **a**	2761.3 ± 38.0 **a**	***NS***
		Female	2362.8 ± 38.8 **b**	2359.0 ± 38.5 **b**	2360.3 ± 38.2 **b**	***NS***
	p		*0.05*	*0.05*	*0.05*	

IGF-2 =insulin-like growth factor-2; 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.

Table 3. The effect of different polymorphisms in 5'UTR of IGF-2 gene on weekly body weight gain in both sexes of Cobb and Hubbard broiler breeds (gram). (Mean ± SE).

Age (days)	Factors		Genotype			*p*
			TT	**TC**	**CC**	
(1-7)	**Breed**	**Cobb**	25.5±1.32 **a**	26.6±1.32 **a**	25.6±1.32**a**	***NS***
		Hubbard	28.0±1.33 **a**	28.5±1.33 **a**	28.3±1.32 **a**	***NS***
	p		***NS***	***NS***	***NS***	
	Sex	**Male**	28.4±1.33a	28.6±1.36a	28.2±1.36	***NS***
		Female	25.7±1.32a	26.5±1.32a	25.6±1.33a	***NS***
	p		***NS***	***NS***	***NS***	
(8-14)	**Breed**	**Cobb**	58.5±4.43 b	60.5±4.41 b	55.4±4.42 b	***NS***
		Hubbard	76.8±4.47 a	78. 7±4.45 a	75.5±4.45 a	***NS***
	p		***0.05***	***0.05***	***0.05***	
	Sex	**Male**	76.62±4.47a	78. 4±4.45a	75. 5±4.45a	***NS***
		Female	58. 5±4.43b	60.6±4.40 b	56.6±4.40 b	***NS***
	p		***0.05***	***0.05***	***0.05***	
(15-21)	**Breed**	**Cobb**	256.6±6.32 b	258.4±6.32 a	257.5±6.35 b	***NS***
		Hubbard	284.8±6.35a	288.5±6.32a	285.15±6.31a	***NS***
	p		***0.05***	***0.05***	***0.05***	
	Sex	**Male**	284.7±6.36a	288. 8±6.32a	285.4±6.32a	***NS***
		Female	256.7±6.30b	258. 2±6.36b	257.5±6.33b	***NS***
	p		***0.05***	***0.05***	***0.05***	
(22-28)	**Breed**	**Cobb**	452.7±11.75a	453.5±11.76a	452. 6±11.75a	***NS***
		Hubbard	460.5±11.74a	464.2±11.75a	463.2±11.74a	***NS***
	p		***NS***	***NS***	***NS***	
	Sex	**Male**	460.4±11.74a	464.2±11.75a	463.2±11.74a	***0.05***
		Female	453.5±11.78a	458.2±11.76a	452.0±11.73a	***NS***
	p		***NS***	***NS***	***0.05***	
(29-36)	**Breed**	**Cobb**	480.7±15.52b	482.5±15.53b	557.3±15.56a	***0.05***
		Hubbard	567.5±15.58a	562.2±15.52a	556.3±15.54a	***NS***
	p		***0.05***	***0.05***	***NS***	
	Sex	**Male**	567.6±15.58a	562.2±15.58a	556.4±15.54a	***NS***
		Female	481.7±15.57b	482.5±15.52b	557.5±15.56a	***0.05***
	p		***0.05***	***0.05***	***NS***	
(37-42)	**Breed**	**Cobb**	486.7±28.52a	483.7±28.50a	486.6±28.52a	***NS***
		Hubbard	437.7±28.52a	439.7±28.50a	438.8±28.52a	***0.05***
	p		***NS***	***0.05***	***0.05***	

	Sex	**Male**	486.7±28.52a	483.7±28.50a	486.7±28.52a	***NS***
		Female	437.7±28.52b	438.7±28.50b	438. 8±28.52a	***NS***
	p		***0.05***	***0.05***	***0.05***	
(43-49)	**Breed**	**Cobb**	608.0±72.62b	630.3±72.62a	607.2±72.62b	***0.05***
		Hubbard	773.3±76.62a	810.3±72.60a	770.0±72.65a	***0.05***
	p		***0.05***	***NS***	***0.05***	
	Sex	**Male**	772.3±76.62a	810.4±72.60a	770.2±72.65a	***0.05***
		Female	608.0±72.62b	630.3±72.62b	607.2±72.65b	***0.05***
	p		***0.05***	***0.05***	***0.05***	

IGF-2 =insulin-like growth factor-2; 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.

0.39 And 1.64 were the allele heterozygosity and allele effective size, respectively. The Chi-square test revealed the absence of the Hardy-Weinberg equation (P≤0.01), showing the existence of allele and genotype frequency transformers between generations. The CC genotype considerably outperformed the TT and TC genotypes in terms of 7th week weight and puberty weight among the genotypes of the study group as shown in table (2) and also in table (3). Growth qualities are among the most significant economic features in poultry, which is why identifying genetic data of growth-related genes in domestic animals is so useful for genetic selection and improvement through marker-assisted selection. In hens, the *IGF* system induces liver glycogenesis and enhances DNA synthesis and tissue growth [3]. *IGF*2 is a polypeptide hormone that regulates the division and differentiation of embryonic cells and is crucial to embryo development. In several animals, the gene is expressed throughout development. Until puberty, the transcriptional activity of the *IGF*2 gene is constant throughout the main embryonic phase, but diminishes in many organs, which influences growth [20]. Using candidate genes is an effective strategy for examining connections between gene variation and economically significant characteristics of domestic animals [21,22,23]. This research investigates the polymorphism of the *IGF*2 gene and its connection with growth parameters in commercial broiler chickens. It is demonstrated that the *IGF*2 gene may be a candidate gene for growth and body characteristics, and its genotype is connected with phenotype, confirming its substantial effect on growth and development [20].Our findings corroborate the findings of [19] who found no connection between the single nucleotide polymorphism of the *IGF*2 gene and growth and feeding parameters. In contrast, they discovered a substantial correlation between these polymorphisms and average daily growth at a certain age. PCR-RFLP and DNA sequencing were used to find polymorphism in the exon 2 region of the chicken *IGF*2 gene in a separate investigation by [13, 14 ,15]. We revealed that the CC genotype had superior 7-week and puberty weight growth records compared to the TT and TC genotypes. Consequently, the current study and prior research indicated that *IGF*2 might be a major gene influencing chicken obesity and could be employed as a genetic marker during the selection process.

CONCLUSION:

The polymorphisms of the chicken insulin-like growth factor 2 (*IGF2*) genes were analyzed in two popular broiler breeds. The results showed that the target locus had two alleles, T and C, with frequencies of 73.3% and 26.7%, respectively. There were three

different genotypes identified, each with a frequency 59.1%, 28.4%, and 12.5%. These genotypes were TT, TC, and CC. Comparison of observed and expected frequencies of several *IGF2* gene genotype variants indicated that commercial broiler breeds (Cobb 500 and Hubbard F-15) did not significantly deviate from Hardy-Weinberg equilibrium (P>0.01). Furthermore, it is demonstrated that 7 weeks of age, birds of the genotype TC had a bigger body mass than those of the genotypes TT and CC also in the weight gain. Researchers found that there was a fair amount of variation in the IGF2 gene, suggesting that it could be used as a genetic marker in chicken selection and breeding.

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