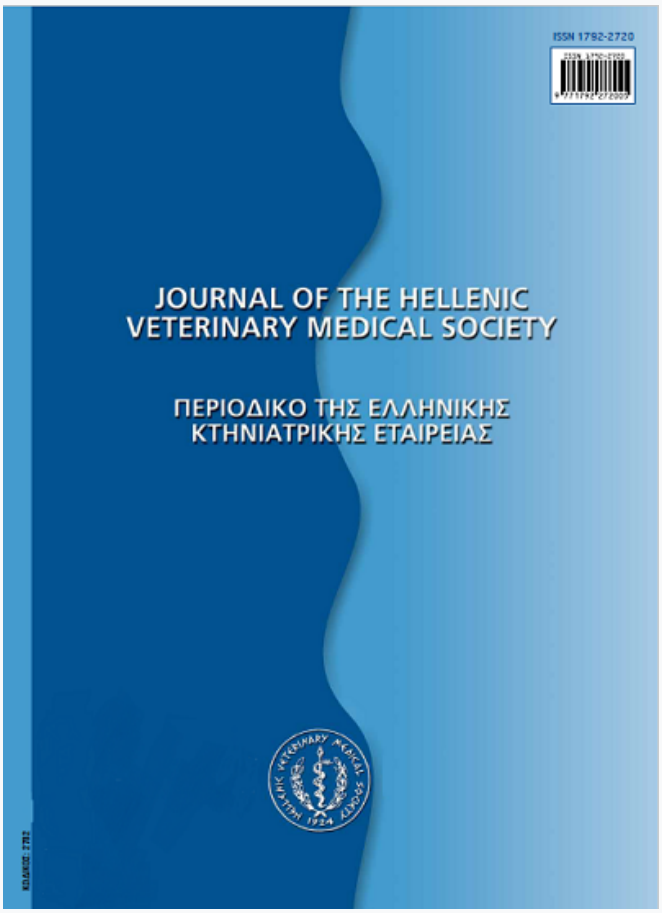
Journal of the Hellenic Veterinary Medical Society

Vol 68, No 2 (2017)



Association of IGF and IGFBP2 gene polymorphisms with growth and egg traits in Atak-S laying hens

H. DAŞ, B. TARIM, S. DEMIR, N. KÜÇÜKKENT, S. CENGIZ, E. TÜLEK, V. AKSAKAL

doi: 10.12681/jhvms.15611

To cite this article:

DAŞ, H., TARIM, B., DEMIR, S., KÜÇÜKKENT, N., CENGIZ, S., TÜLEK, E., & AKSAKAL, V. (2018). Association of IGF and IGFBP2 gene polymorphisms with growth and egg traits in Atak-S laying hens. *Journal of the Hellenic Veterinary Medical Society*, *68*(2), 237-242. https://doi.org/10.12681/jhvms.15611

J HELLENIC VET MED SOC 2017, 68(2): 237-242
ΠΕΚΕ 2017, 68(2): 237-242

Research article
Ερευνητικό άρθρο

Association of IGF and IGFBP2 gene polymorphisms with growth and egg traits in Atak-S laying hens

Da H.[1*], Tarım B.[2], Demir S.[2], Küçükkent N.[3], Cengiz S.[3], Tülek E.[2], Aksakal V.[4]

[1] *Gümü hane University, Department of Veterinary,* Gümü hane, TURKEY

[2] *Ankara Poultry Research Institute, Ankara, TURKEY*

[3] Gümü hane U*niversity, Department of Medical Laboratory Techniques, TURKEY*

4 Gümü hane University, Kelkit JTC, Department of Animal and Plant Production, Gümü hane, TURKEY

**Correspondence: Huseyin DA , Gümü hane University*

ABSTRACT. Atak-S laying hens are a high-performance hybrid obtained by crossing of the Rhode Island Red (RIR) X the Barred Plymouth Rock (BR) and are being produced in the Ankara Poultry Research Institute since 1997. Phenotypic and genetic improving studies are continuous for this hybrid. In this study three different SNPs, two from *IGF*1 and one from *IGFBP2* genes, were examined in 150 Atak-S chickens. Genetic association of SNPs were compared to body weight and egg number till 32 weeks of age, body weight at sexual maturity, age at sexual maturity and also egg quality traits such as egg shell breaking strength, shell thickness, Haugh unit, albumen index, yolk index and shape index were statistically analyzed. Only *IGF1*(a) locus was in agreement with Hardy-Weinberg equilibrium, while, the rest of the loci were not. As a result of the comparisons performed to the three SNPs, it was determined that there was a significant association ($P<0.05$) between the T364C haplotypes of the *IGF1*(b) locus and body weight at 32 weeks of age, but there was not any association to the other traits.

Keywords: Atak-S, *IGF*, *IGFBP2*, single nucleotide polymorphism

Correspondence:
JTC, Department of Veterinary,
Gümü hane, 29000 TURKEY
+904562331000,
huseyindas@hotmail.com

Date of initial submission: 7.1.2016
Date of revised submission: 3.6.2016
Date of acceptance: 17.6.2016

Ημερομηνία αρχικής υποβολής: 7.1.2016
Ημερομηνία αναθεωρημένης υποβολής: 3.6.2016
Ημερομηνία αποδοχής: 17.6.2016

INTRODUCTION

Atak-S laying hens are a high-performance hybrid obtained by crossing of the Rhode Island Red (RIR) X the Barred Plymouth Rock (BR) produced in the Ankara Poultry Research Institute since 1997 (Durmu and Kamanli, 2015). It's one of the three lines of laying hens developed in the Turkey. Further improvement of this hybrid will be useful for the Turkey's economy, so the studies are continuing.

The quantitative characters most of which have economic importance in the laying hens show a continuous variation and occur under complex mechanisms with the effect of many genes such as Prolactin (PRL), dopamine D1 receptor (DRD1) etc… Detecting these genes is a critical aim in modern selection techniques. Investigation of direct effects of candidate genes on certain traits is a reliable method but sometimes it may be time and effort consuming way.

The chicken insulin-like growth factor family comprises two genes named *IGF1* and *IGF2*. *IGF1* is a polypeptide composed of 70 amino acids with a molecular weight of 70 kda. The gene extends to more than 50 kb, with four exons and three introns, located in chromosome 1. Its role on glucose homeostasis, amino acid, protein synthesis, muscle development, and feed conversion are known (Kocamis et al., 2001, Duclos et al., 1999). These effects are due to its function as a growth hormone mediator or local autocrine or paracrine growth stimulator (Pollak et al. 2004). *IGF1* is expressed in many tissues such as liver, brain, heart and skeletal muscles. The *IGF*-binding protein2 (*IGFBP2*), however, which is located in the 7th chromosome has a length of 38 kb with 4 short exons and three long introns. The *IGFBP2*, whose the most important regulator is growth hormone, forms a polypeptide with a size of 275 amino acids. In this family, six *IGFBP* proteins have been identified in the mammals, while five proteins have been identified in the chickens so far (Daza et al., 2011). After the *IGF1* is released to the blood circulation, it binds to its *IGFBP* receptor which is expressed in various tissues.

Despite all this knowledge, the roles of the *IGF* and the *IGFBP* proteins on the metabolism and the availability thereof in the selection for the livestock have not been understood well. The results are contradictory in relation to whether *IGF* genotypes directly affect the growth traits in the chickens or not. For instance, while there are studies reporting that the *IGF1* does not induce growth (Huybrechts et al. 1992, Nagaraja et al. 2000), there are more recent studies reporting that it has a relation to the growth properties (Wang et al. 2004, Moe et al. 2009). Moreover, although selection of the broilers in the body weight (bw) for long years resulted to increase in expression of serum *IGF1* mRNA (Beccavin, 2001), no correlation between the serum *IGF1* concentration level and bw was reported (Yun et al., 2005). These results leave open the question whether the *IGF1* directly affects the growth or increases along with the growth.

Therefore, these genes are required to be thoroughly examined on different strains of chickens. SNPs studied in this research were used in different researches for different breeds to examine their relationship to productivity (Bian et al. 2008, Li et al. 2008, Sudaryati et al. 2013).

The objective of this study is both to seek an answer to the aforementioned questions and to examine the availability of these genes in the selection of Atak-S chickens by comparing the polymorphisms of these genes to the performance values up to 32 week.

MATERIALS AND METHODS

Experimental chickens and housing conditions

This study was performed in Ankara Poultry Research Institute which is the development and production center of Atak-S chickens. 150 chickens were used in this study and their phenotypic and genetic properties were determined. The study started from the first day of hatching and continued until completing 32 week of age. Chickens were raised together in floor and moved to the multilayer individual cages at the 12 week of age. All chickens were raised in the same room and under the same conditions, and were fed a commercial corn-soybean-based diet and fresh water ad libitum. Chickens were fed with four different rations, starter (19% protein and 2900 kcal/kg ME), chick starter (18% protein and 2800 kcal/kg ME), chick grower (16% protein and 2700 kcal/kg ME) and layer (17% protein and 2700 kcal/kg ME) from 0 to 4, 5 to 12, 12 to 17 and 18 to 32 week of age, respectively. Lighting period and intensity were applied as reported by Durmu and Kamanli (2015). This study was approved by the ethical committee of Ankara Poultry Research Institute with number of 2015/01.

Phenotypic measurements

Bw measurements were made every 4 weeks. Age and weight at sexual maturity were recorded day by day. Egg productions were recorded daily from reaching the sexual maturity to the 32nd week. The measurements of egg quality traits were made by taking the average of eggs obtained over consecutive three days at 28 and 32 week of age. The eggs collected to examine the quality traits were kept at 18-20 °C for 24 hours and then cracked and their properties were detected. Besides, during the detection of the internal quality, the examination of each egg was paid attention to not exceed 10 minutes. Egg weights were measured on a precision balance and recorded. Albumen and yolk height were detected with Futura® height gauge while the widths thereof were detected with digital compass electronically. Albumen and yolk index was determined with the method reported by Uyanık et al. (2002). Haugh unit, on the other hand, was calculated from the formula of $Haugh\ Unit = 100\ Log\ (H + 7.57 - 1.7G^{0.37})$ using albumen height and egg weight. In this formula, H represents the albumen height in mm while G represents egg weight in gram. Shell thickness was measured by being separated from its membranes with Mitutoyo® digital micrometer. Egg shell breaking strength was measured in g/cm^2 using Futura® resistance meter.

DNA extraction and PCR assay

Blood was taken from the chickens from the vein of the bottom of the wing at 16 week of age. For *IGF1*(a), *IGF1*(b) and *IGFBP2*, the methods reported respectively by Bian et al. (2008), Li et al. (2008) and Sudaryati et al. (2013) were modified and used. Briefly, genomic DNA was extracted using Qiagen DNeasy Blood and Tissue kit from venous blood collected in EDTANa2-coated tubes. By measuring optical density at 260 nm (OD260) absorbance and OD260/280 ratio and by running agarose gel electrophoresis, the concentration and purity of DNA preparations were verified. Working dilutions of extracted DNA were prepared at a concentration of 25 ng/µg for each chicken. The reaction mixture for a single sample included 2 µl DNA, 2.5 µl 10× PCR buffer, 0.5 µl of dNTP mix, 1 µl primers forward and reverse, 1 U TaqDNA polymerase (Qiagen, TaqPCR Core Kit) and 18 µl ultrapure water. The final volume of the reaction was 25 µl. The PCR conditions were started with 94°C-5 min, followed by 35 cycles at 94°C-30s for denaturation, annealing at 51°C for *IGF1*(a), 53°C for *IGF1*(b), 60°C for *IGFBP2* for 60s, at an extension step, at 72 °C-1 min, with a final extension step of 72 °C-10 min. The information of the primers used are given in Table 1.

Digestion PCR product and Screening

HinfI restriction enzyme was used to detect A366C SNP in *IGF1*(a) gene, PstI restriction enzyme was used to detect C364T SNP in *IGF1*(b) gene and Eco72I to detect C1032T SNP in *IGFBP2* gene. The PCR products were digested overnight at 37°C using 10 U of the restriction enzymes. The digested products were run by horizontal electrophoresis (70 volts, 40 min) in 2,5% agarose gels in 1×TBE containing 1.0 µM of ethidium bromide. 100 bp DNA Ladder (Thermo Fisher Scientific) was employed to determine the sizes of fragments. Individual PCR-RFLP fragment sizes were determined by visualizing with UV transilluminator (WiseUv® WUV-L50).

Statistical Analyses

Statistical analyses were executed using SPSS 20 software. To check whether the data were appropriate to the parametric method, homogeneity of variance and normal distribution tests were applied.

Table 1. Properties of the primers and digestive enzymes of the SNPs examined.

Gene	Primer Sequences	Enzyme	SNP	Length	Temp.
IGF1 (a)	F 5 - CACAGCCACCCGAAAGT- 3 R 5 - AGAAATCACAAAAGCAGCAC- 3	HinfI	A366C	542 bp	51 °C
IGF1 (b)	F 5 - GACTATACAGAAAGAACCCAC - 3 R 5 - TATCACTCAAGTGGCTCAAGT - 3	PstI	C364T	621 bp	53 °C
IGFBP2	F 5 - TTTGGTTGAGTCCTAGGCTTG - 3 R 5 - GGCGTACTACACTGCAGAGG - 3	Eco72I	C1032T	527 bp	60 °C

Then, the data were analyzed using one-way ANOVA comparing genotypes to phenotypes. Y=μ+G+e mathematical model was employed, where Y is trait value; μ is overall population mean; G introduce the effect of genotype and e the residual random error. Statistical significance level was determined as P<0.05. Frequencies of distribution alleles and Hardy-Weinberg equilibrium (HWE) were evaluated using the chi-square test.

RESULTS

Genotype frequencies determined for *IGF1* and *IGFBP2* genes and testing Hardy-Weinberg equilibrium

IGF1(a) locus contains SNP A366C in the 5'-UTR. After PCR products were hydrolyzed with *Hinf*I overnight, they were separated by electrophoresis and observed for the 3 different combinations of band patterns. CC, AA and AC genotype exhibited fragments with a length of 542 bp, 428 bp, and 428-542 bp, respectively. Numbers of the individuals of each genotype, gene frequencies and chi-square results were denoted in Table 2. Obtained genotype frequencies were in good agreement with expectations of HWE. After treated *IGF1*(b) with *Pst*I, fragments in length of 364-257, 364-257-621, and 621 bp were obtained for CC, CT, and TT genotype respectively, from the amplicon with a length of 621 bp. In terms of HWE, the observed genotypes were different from the expected values. Namely, the population was not in agreement with HWE. For *IGFBP2* gene, SNP C1032T is in the 2nd intron. Fragments with a length of 477, 477-527, and 527 bp were obtained for CC, CT and TT genotype respectively, after treating PCR products with *Eco*72I. The population is in Hardy-Weinberg disequilibrium at this locus.

Phenotypic values and its association with SNP loci

In this study, bw values from 4 to 32 weeks, that is a performance factor of the laying hens were examined and the results were denoted in Figure 1, 2 and 3 for *IGF1*(a), *IGF1*(b) and *IGFBP2*, respectively. The results of the bw at sexual maturity, egg number at 32 week of age and age at sexual maturity corresponding to *IGF1* and *IGFBP2* genes were displayed in figure 4, 5 and 6. Egg shell breaking strength, Haugh unit, shell thickness, albumen index, yolk index, shape index were also displayed in Table 3. For *IGF1*(b), bw values at 32 week were obtained as TC>CC>TT (P<0.05). Apart from that, there was not any growth and egg traits which present a significant association with the 3 loci (P>0.05).

DISCUSSION

The traits such as growth properties and egg quality exhibit a continuous variation and are determined by many genes. Since the interaction of gene network is very complex, the genes of interest might not exhibit the expected effect in each genotype (Jaenisch and Bird, 2003). Therefore, the effects of polymorphisms are required to be tested with various hypotheses in different species and strains. Another important point is that a protein in an organism has sometimes sever-

Table 2. Gene frequencies of the loci, expected and observed genotype frequencies and chi-square results.

Gene	Gene frequency		Observed and expected frequencies of Genotypes		X^2
IGF1 (a)	A	0,46	AA	29 (32)	0,97
	C	0,54	AC	80 (74)	
			CC	41 (44)	
IGF1 (b)	T	0,43	TT	46 (29)	33,4*
	C	0,57	TC	39 (74)	
			CC	65 (47)	
IGFBP2	T	0,26	TT	5 (10)	4,8*
	C	0,74	CT	69 (58)	
			CC	76 (81)	

* Expected and observed values are different; locus is not in agreement with HWE. (P<0.05)

Figure 1. The bw values from 4 to 32 weeks belonging to IGF1(a) locus are demonstrated

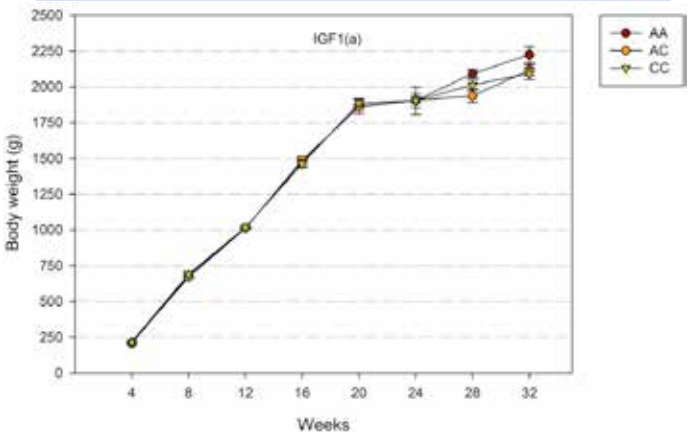


Figure 2. It displays the bw values from 4 to 32 weeks belonging to IGF1(b) locus.

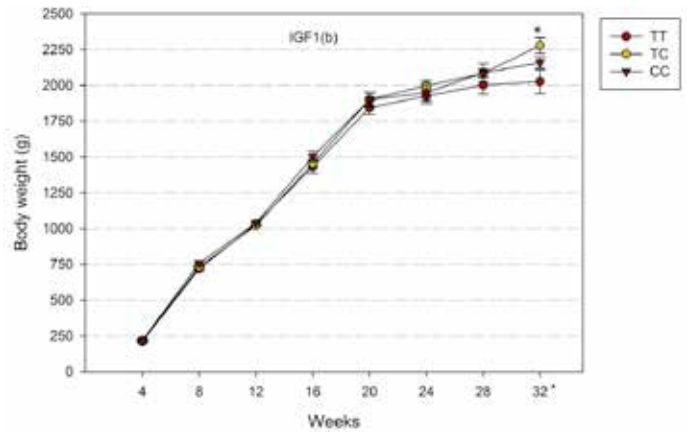


Figure 3. The bw values from 4 to 32 weeks belonging to IGFBP2 locus are demonstrated.

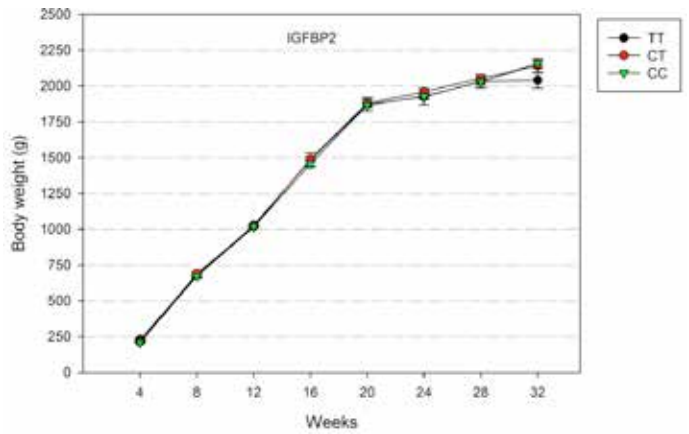


Figure 4. Bw at sexual maturity results corresponding to IGF1 and IGFBP2 genes are shown. None of the genotypes provide a significant difference than the others (P>0.05).

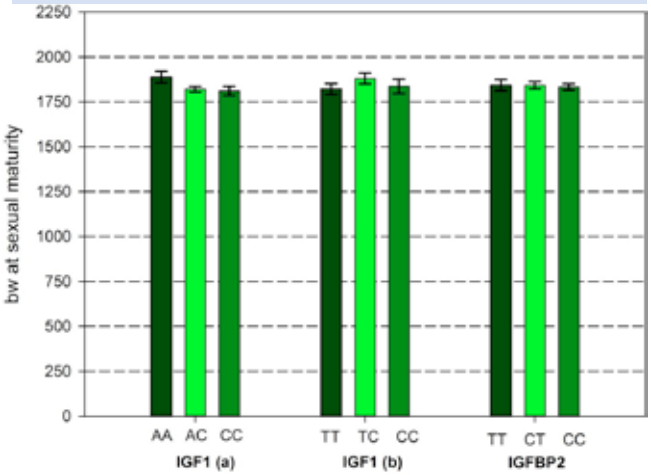


Figure 5. It demonstrates the results of egg number until 32 week of age, belonged to IGF1 and IGFBP2 genes. None of the genotypes provide a significant difference than the others (P>0.05).

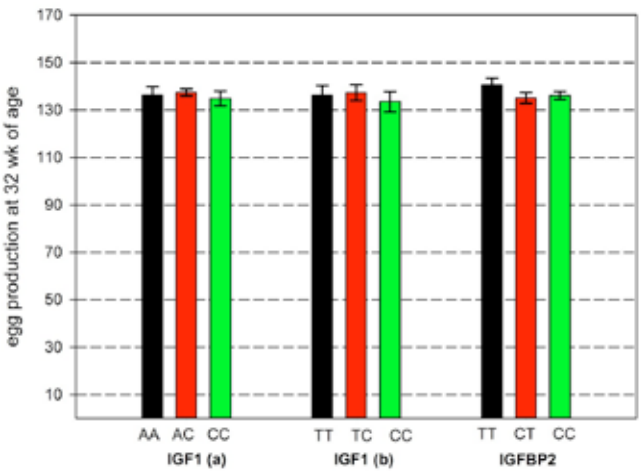


Figure 6. Age at sexual maturity results corresponding to IGF1 and IGFBP2 genes are displayed. Alleles do not exhibit a significant difference by the ANOVA analyses performed between genotype and phenotype values (P>0.05).

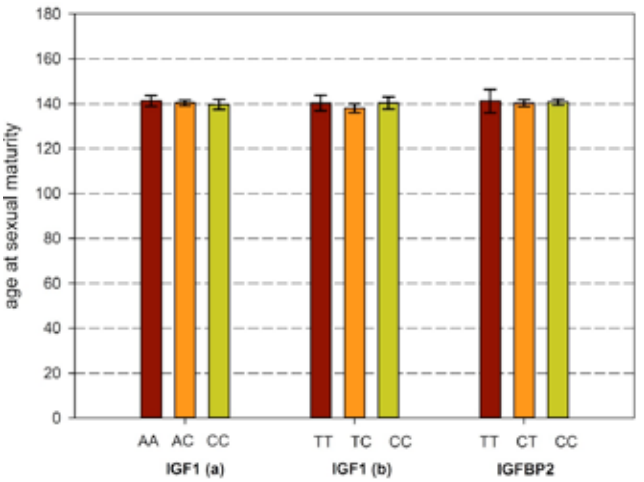


Table 3. The results of the egg quality traits determined according to the loci.

		Egg weight	Break strength	Haugh unıt	Shell thick-ness	Albumen index	Yolk index	Shape index
IGF1 (a)	AA	62,0±0,6	4252,1±205,8	79,8±2,5	339,4±6,9	8,4±0,5	47,7±1,3	75,4±0,4
	AC	59,3±0,5	4355,1±103,8	78,6±1,4	340,3±3,6	7,8±0,2	47,1±0,7	75,6±0,4
	CC	60,0±0,7	4281,2±153,4	76,8±2,7	350±8,8	7,9±0,3	48±1,3	74,6±0,4
	Total	60,1±0,4	4315,9±79,4	78,4±1,1	342,5±3,2	8±0,1	47,5±0,5	75,3±0,2
	P	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
IGF1 (b)	TT	60,0±1,1	4284,2±228,7	75,2±3,8	350,5±3,9	7,4±0,5	49,6±1,8	75,8±0,8
	TC	61,1±0,7	4434,1±308,3	74,1±3,7	351±9,8	7,6±0,4	49,4±2,2	75,1±0,7
	CC	60,0±1,4	4254,6±196	76,6±2,8	345,3±9,4	7,7±0,4	48,4±1,2	75,5±0,6
	Total	60,5±0,5	4303,2±131,9	75,6±1,9	348,3±4,9	7,6±0,2	49±0,9	75,5±0,4
	P	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
IGFBP2	TT	60,2±1,4	4342,4±182	80±1,6	331±12,6	7,7±0,2	44,7±1,2	76,6±0,6
	CT	60,2±0,5	4360±119,1	78,2±1,6	346,8±4,5	8±0,2	47,8±0,8	75,5±0,3
	CC	60,0±0,5	4308±106,5	77±1,7	345,3±4,6	7,7±0,2	47,3±0,7	75±0,3
	Total	60,2±0,3	4334,4±76,5	77,7±1,1	345,3±3,1	7,8±0,1	47,5±0,5	75,3±0,2
	P	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

al functions, so it might be influencing many traits, a phenomenon that is known as pleiotropic effects (Foster et al., 2004). Hence, the effects of some polymorphisms of the *IGF1* and the *IGFBP2* genes on egg traits as well as growth traits have been already analyzed. Candidate gene approach is also an important method which is frequently used in determining the function and effects of genes (Akyüz et al, 2013). SNPs which are the most common mutation type in the genome have a potential effect of changing the expression and products of genes. Therefore, they can directly affect the phenotype or can function as a marker by means of linkage with another affecting locus. As will be stated below, different studies were examined the *IGF* gene loci as candidates markers for the selection of various breeds. However, no similar studies in Atak-S laying hens have been conducted; Atak-S laying an economically important strain for economy of Turkey and the effects of *IGF* genes on the egg traits were not sufficiently introduced yet, thus it is unclear. In this study, the polymorphisms of *IGF1* and *IGFBP2* genes and the usability thereof in the selection were examined with PCR-RFLP method. Haplotypes of SNPs were compared using GLM procedures to bw from 4 to 32 week, bw at sexual maturity, egg number at 32 week of age, age at sexual maturity and also egg quality traits such as egg shell breaking strength, shell thickness, Haugh unit, albumen index, yolk index, shape index.

IGF1(a) was in agreement with HWE, while *IGF1*(b) and *IGFBP2* genes were not. The reason for the disequilibrium may be the effects of the selection of this hybrid. *IGF1*(a) of them was first reported to correlate with egg and shell weight by Nagaraja et al. (2000). However, Moe et al. (2009) compared *IGF1*(a) with bw in Asian native chickens and reported that AA genotype has a high bw. Zhou et al. (2005) showed that this locus was in relation with bw in broilers while Promwatee and Duangjinda (2010) reported same results in laying hens. In this study, on the other hand, no association between *IGF1*(a) locus and productivity of Atak-S chickens was encountered. Similar to our results, Babayi et al. (2014) reported that it was also not associated with bw. These different reports suggest that the more analytical studies in various disciplines are needed to give explanations about the basis of these differences.

Seo et al. (2001) reported that although TC haplotype was much higher in *IGF1*(b) locus at 30 week of age, this difference was disappeared in the other months.

al functions, so it might be influencing many traits, a phenomenon that is known as pleiotropic effects (Foster et al., 2004). Hence, the effects of some polymorphisms of the *IGF1* and the *IGFBP2* genes on egg traits as well as growth traits have been already analyzed. Candidate gene approach is also an important method which is frequently used in determining the function and effects of genes (Akyüz et al, 2013). SNPs which are the most common mutation type in the genome have a potential effect of changing the expression and products of genes. Therefore, they can directly affect the phenotype or can function as a marker by means of linkage with another affecting locus. As will be stated below, different studies were examined the *IGF* gene loci as candidates markers for the selection of various breeds. However, no similar studies in Atak-S laying hens have been conducted; Atak-S laying an economically important strain for economy of Turkey and the effects of *IGF* genes on the egg traits were not sufficiently introduced yet, thus it is unclear. In this study, the polymorphisms of *IGF1* and *IGFBP2* genes and the usability thereof in the selection were examined with PCR-RFLP method. Haplotypes of SNPs were compared using GLM procedures to bw from 4 to 32 week, bw at sexual maturity, egg number at 32 week of age, age at sexual maturity and also egg quality traits such as egg shell breaking strength, shell thickness, Haugh unit, albumen index, yolk index, shape index.

IGF1(a) was in agreement with HWE, while *IGF1*(b) and *IGFBP2* genes were not. The reason for the disequilibrium may be the effects of the selection of this hybrid. *IGF1*(a) of them was first reported to correlate with egg and shell weight by Nagaraja et al. (2000). However, Moe et al. (2009) compared *IGF1*(a) with bw in Asian native chickens and reported that AA genotype has a high bw. Zhou et al. (2005) showed that this locus was in relation with bw in broilers while Promwatee and Duangjinda (2010) reported same results in laying hens. In this study, on the other hand, no association between *IGF1*(a) locus and productivity of Atak-S chickens was encountered. Similar to our results, Babayi et al. (2014) reported that it was also not associated with bw. These different reports suggest that the more analytical studies in various disciplines are needed to give explanations about the basis of these differences.

Seo et al. (2001) reported that although TC haplotype was much higher in *IGF1*(b) locus at 30 week of age, this difference was disappeared in the other months. So except for this week, they reported that it didn't have any significant relation with bw. In our study, similarly, TC haplotype was higher than TT and CC haplotypes in terms of bw at 32 week of age, which means that results of these two studies are similar. In contrast to these results, Amills et al. (2003) reported that this locus was not related to bw at any week. Li et al. (2010) examined the same locus in terms of egg quality and reported that this locus was only associated with egg shell thickness and egg weight. There are also studies reporting that it is associated with total egg numbers (Kim et al., 2004; Li H. F et al., 2009). According to our results, *IGF1*(b) locus has no effect on egg traits and numbers. *IGFBP2* locus had not also any effect on given phenotypic traits likewise to the other loci. Similar to our findings, Sudaryati et al. (2013) controlled the activity of the same locus on Kampung chickens and reported that it didn't have any significant association with bw. However, Li et al. (2006) examined this locus on broilers, and reported to have had significant differences in terms of bw in all weeks. Khadem et al. (2010) examined a different locus of *IGFBP2* on Mazandaran chickens and reported that it didn't have an association with bw and egg number.

As reported by Promwatee and Duangjinda (2010), while SNPs in *IGF1*, cGH, and *IGFBP2* were associated significantly with growth traits for one strain, this association was insignificant for another breed. Li W. et al. (2009) also similarly reported that *IGF1*(b) locus was associated with bw in some strains and not associated with the others. The reason for this may be based on that SNP which are thought to affect a character are in linkage with other mutation which has real effect on this character so, if this mutation is close to a SNP, they are transferred together in just some strains. A second possibility is that the effects revealed by a mutation might be discrete due to complex protein and signal networks in one organism, so the phenotypes might be different.

CONCLUSIONS

With the association studies of the 3 SNP loci, it was determined that there is a significant association between TC genotypes of the *IGF*(b) locus and bw at 32 weeks of age ($P<0.05$), but there wasn't any association for other traits. As a conclusion; except for *IGF*(b) locus, the studied SNPs in *IGF1* and *IGFBP2* have not any relation to egg and growth traits in

Atak-S chickens; it is still early to say whether *IGF* correlated to genes are associated with the traits of other economically important laying hens.

ACKNOWLEDGEMENTS

This project was supported by Gümü hane University with the project no. 14.B0121.02.01. Feeding and other costs of the chickens were supplied by the Ankara Poultry Research Institute. We thank both institutes for their support.

CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

REFERENCES

Akyüz B, Arslan K, Bayram D, I can K M (2013) Allelic frequency of kappa-casein, growth hormone and prolactin gene in Holstein, Brown Swiss and Simmental cattle breeds in Turkey. Kafkas Univ Vet Fak Derg 19(3):439-444.

Amills M, Jimenez N, Villalba D, Tor M, Molina E, Cubilo D, Estany J (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. Poultry Sci 82(10):1485-1493.

Babayi MS, Seyedabadi H, Gorbani A, Zarghami N (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.

Beccavin C, Chevalier B, Cogburn LA, Simon J, Duclos MJ (2001) Insulin-like growth factors and body growth in chickens divergently selected for high or low growth rate. J. Endocrinol 168:297-306.

Bian L H, Wang S Z, Wang QG, Zhang S, Wang YX, Li H (2008) Variation at the insulin-like growth factor 1 gene and its association with body weight traits in the chicken. J Anim Breed Genet 125(4):265-270.

Daza DO, Sundström G, Bergqvist CA, Duan C, Larhammar D (1999) Evolution of the insulin-like growth factor binding protein (IGFBP) family. Endocrinol 152:2278-2289.

Duclos MJ: Regulation of chicken muscle growth by insulin-like factors. Trends in comparative endocrinology and neurobiology. Ann. NY Acad. Sci 839:166-171.

Durmu , Kamanlı S (2015) Effects of Cold and Heat Stress on Egg Quality Traits of a Newly Developed Native Hybrid Layer. Turkish Journal of Agriculture-Food Science and Technology 3(6):444-447.