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Association of G143831A SNP Of Growth Hormone Gene And Some Productive Traits, Physiological Parameters Of Laying Hens.

Bassam G M Al-khatib[1]*, Alan A Noori[2], and Ibtisam Q Abdul kareem[2].

[1]Department of higher studies, University of Baghdad, Iraq
[2]Department of Animal Production, Agricultural Faculty, University of Baghdad, Iraq

ABSTRACT

This study was carried out in the Poultry farm / college of agriculture / university of Baghdad. This experiment aimed to detect the various genotypes of GH gene in the laying hens and observe its association with some productive traits and physiological parameters. When using of Eco RV restriction enzyme three genotypes of the third intron of GH gene were found homozygous (wild) AA, heterozygous AB and homozygous (mutant) BB and the differences between the various genotypes were highly significant (P<0.01). There were no significant effect showed of the various genotypes on the physiological parameters (glucose, cholesterol, triglyceride, low density lipoprotein, total protein) and all the productive traits, accept the weekly egg production at the third week of production, the genotypes AA had a significant effect (P<0.05) followed by AB then BB (5.92, 5.12 and 4.58) respectively.

Keywords: hormone gene, laying hens

Corresponding author

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INTRODUCTION

Economic integration is the aim of the developed countries and the animal production field have the crucial role of this aim, poultry industry one of the important branches of animal production provides (egg, white meat, hatching egg), and all these products are healthy because of its low content of cholesterol and lipids that due to many human health problems like heart diseases, hyperplasia and high levels of cholesterol and triglyceride of blood plasma (Kaya and Yıldız, 2008.; Kadlec, 2011). Egg represented an important source of protein and its biological value amounted 100%. All these benefits of egg and white meat of chicken led to high interesting from global companies to develop the broiler and layer strains to reach the optimal product performance and decreasing of the production period throw modern selection programs depends on the molecular genetic technique as genetic markers (RFLP,AFLP,SSR,SSCP) and gene cloning in candidate genes or somatotropic axis genes like (GH, GHR, IGF1- ,IGFR-1-2,Leptin,Insulin)(Li *et al*. 2010; Anh *et al*., 2013) . These genes affect significantly on the quantitative traits as meat, egg production and any mutations or genetic changes in the nucleotide structure may be led to positive or deleterious effect on chicken productive performance. Growth hormone gene and some candidate genes are the most important groups of genes that encode many functional hormones affect directly on productive and physiological performance by its important roles in embryonic development to maturity and productive stage growth and metabolism of laying hens (Kulibaba and Tereshchenko, 2015). This study aimed to determine the GH gene polymorphism and its association with productive performance and some physiological parameters of hens in Iraq.

MATERIAL AND METHODS

Five ml of blood was collected from the brachial vein of 160 layer hens under the study. These samples were collected in EDTA tubes and kept in the freezer (-18 ºC) for DNA extraction by using DNA extraction kit (Promega, USA. before DNA extraction blood volume was reduced to 20 microliters and cell lysis buffer increased to 500 microliters because of all the blood cells of chicken are nucleated and contained DNA and protein levels in chicken blood higher than in mammals blood(Al-khatib and Al-hassani,2016). The primers were supplied by Alpha DNA/Canada, as lyophilized powder of different picomols concentrations F-**5'**TCCCAGGCTGCGTTTTGTTACTC**3'** and R-**5'** ACGGGGGTGAGCCAGGACTG **3'** gene bank (AY461843) according to (Nie *et al*., 2005)

PCR reaction and Enzyme digestion

The PCR reaction was performed in 0.2ml tubes by mixing master mix reagents in a final volume of 20 µl. The amplification was performed in a TECHNE (T-C 5000) thermal cycler and the reaction mixture was prepared according to the procedure that suggested by the manufacture company (BIONEER, Korea) using 75-90 ng/ µl of DNA and 0.8 µl of primers and then complete the PCR reaction volume to 20 µl with distilled water finally reaction mixture vortexes thoroughly. PCR mixture without DNA template was used as a negative control. Thermal cycle with the following profile: Initial denaturation at 94°C for 4 minutes, 35 cycles of 94 °C for 30 seconds,54 °C for 30 seconds,72°C for 30 seconds and a final elongation at 72 °C for 5 minutes. PCR products (8µ) were digested with 3 units of *Eco*RV restriction enzyme at the 37 °C overnight. A Restriction pattern was visualized in a 1.5% agarose gel electrophoresis stained with Ethidium bromide.

Productive traits

Egg productive weekly per hen was calculated for 100 days, according to Nair and Ghadoliya (2000). Egg quality traits were measured (yolk and albumen height, shell thickness, yolk width, width and length egg, egg weight, Haugh unit) according to Stadelman and Cotterill (1995).

Physiological parameters

Some of physiological parameters were measured in serum by collecting 5ml of blood in anticoagulant tubes, then isolate the serum according to (Henry *et al*., 1974).After isolating the serum some of physiological parameters were measured (glucose, cholesterol, triglyceride, low density lipoprotein, total protein) respectively, using (Accent 200 automated biochemical analyzer of the company Cormay, Poland) apparatus.

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Statistical analysis

Data were statistically analyzed using statistical analysis system program – SAS (2012) to study the effect of GH gene polymorphisms in various traits and compared the significant differences between the averages using the Duncan test (1955) polynomial. The Mathematical model for detecting the GH gene polymorphisms in traits studied

Yij= M+Ai+eij

RESULTS AND DISCUSSION

PCR-RFLP analysis

Polymerase Chain Reaction (PCR) amplified regions, which showed a molecular weight of 429 bp, represents the region of the growth hormone gene. This technique was used with primers for growth hormone gene, according to Nie et al, 2005 . To detect the PCR product, DNA ladder (100-1000) bp was used and the gel was photographed by a digital camera. The same PCR product size was obtained by Nie et al.,2005 then the PCR products which underwent restriction digestion with *Eco* RV enzymes (GAT/ATC) to detect G143831A SNP in the third intron of growth hormone gene and it was able to cut at this position only when SNP is present (when G convert to A).

1. Wild type AA: No cleavage of the whole 429 bp segment by *Eco* RV.
2. Heterozygous AB: *Eco* RV was cut the sequence to show three fragments in agarose gel electrophoresis (429 bp ,295 bp and 134 bp).
3. Homozygote BB: *Eco* RV was cut the sequence to show two fragments in agarose gel electrophoresis (195 bp and134 bp).

TACACCAACA	AAAACTCCCA	GGCTGCGTTT	TGTTACTCAG	AAACCATCCC
AGCTCCCACG	GGGAAGGATG	ACGCCCAGCA	GAAGTCAGTA	AGTTGTCTCC
CCTGGGTAAA	CACAGCACTG	TTTTATGGAA	CAGAGGGTCT	CCACGTGGTA
TCAGTCCCGA	GAAGGAGAAA	TGCCTTCTTA	CTTTTCACAC	CCTGCATGCA
GAAAGACACG	GGTTGGGCAG	TAAATCATAT	TCCCACCCTA	AATAAAGTCC
TAAAAAAACA	GGCTCGAGTC	TGAGTGGTGG	TGCTCAGCTT	ACAGAGCTGC
CTCTGGGCTG	CTTCAGGGAG	AGCAGGGCAT	GCAGCAGCAC	TGCAGAACAC
CTCACCTGCA	CAGCTCTGAA	ATCCCTTTGT	CATTTCAGGA	CATGGAGCTG
CTTCGGTTTT	CACTGGTTCT	CATCCAGTCC	TGGCTCACCC	CCGTGCAATA

Figure 1: Implicon length and primers linke sites in GH gene of ckicken

The results of the present study are similar with previous study of Nie *et al.*, (2005) and Lei *et al*., (2007) on the Chines chicken.

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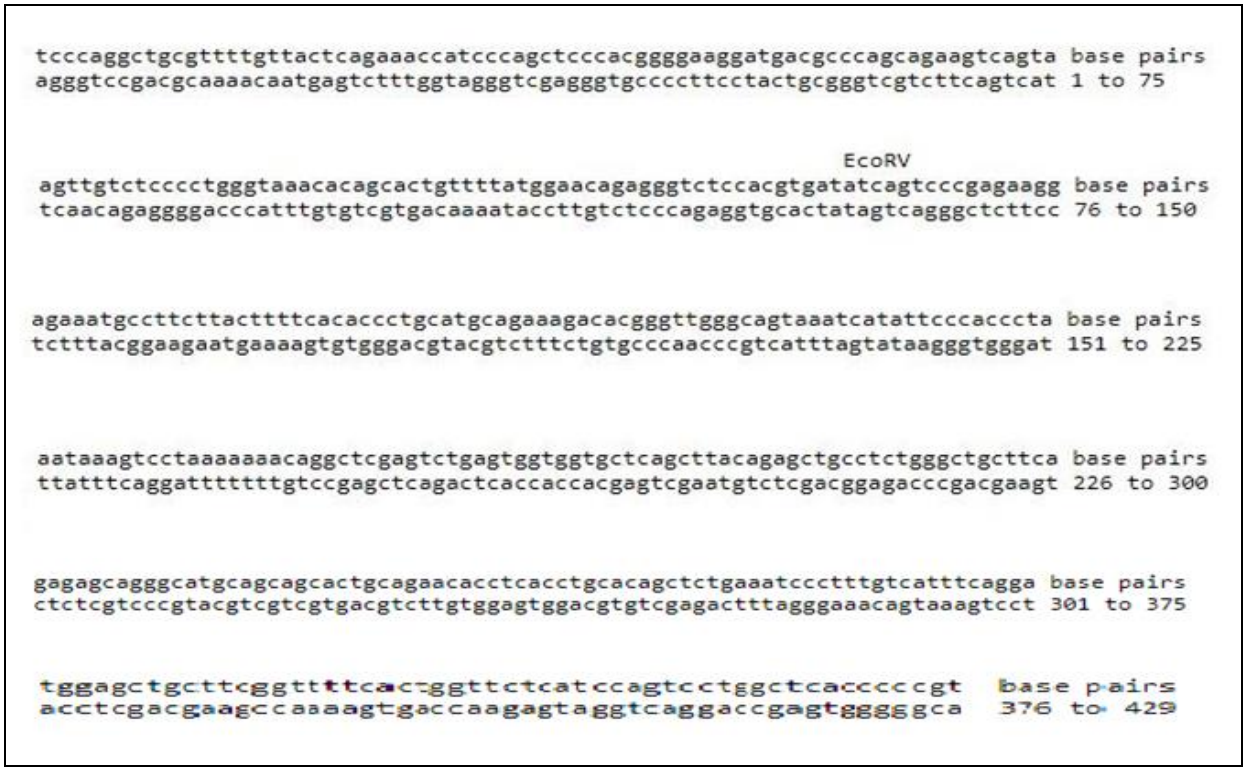

Figure 2: Recognition site of the endonuclease restriction enzyme *Eco* RV on the PCR product

Genotype distribution and allele frequency of GH gene

Table 1 results showed high significant differences (P<0.01) between the various genotypes of GH gene and the heterozygous AB had the highest percentage amounted 0.55, followed by BB then AA genotypes amounted 0.35 and 0.10 respectively and the B allele had a highest significant superiority (P<0.01) over A allele 0.63 , 0.37 respectively. These results are similar with the previous study of (Al-khatib and Al-hassani, 2016) on the third intron of GH gene in broiler chicken.

Table 1: Genotype distribution and allele frequency of GH gene polymorphisms

(%)	No.	Genotype
35.00	70	BB
55.00	110	AB
10.00	20	AA
% 100	200	Total
** 12.663	–––	Chi-square value (χ^2)
Allele frequency		
0.63		allele B
0.37		allele A
) **P<0.01 (		

Effect of GH gene polymorphisms on some physiological parameters

Table 2 revealed that no significant effect of different genotypes of GH gene and the plasma physiological parameters. The un-translating region like introns may be led to this non-significant effect on the physiological parameters (Fedorova and Fedorov, 2003), on the other hand these physiological traits are different from the quantitative traits that affect by many genes and the QTL regions in the chicken genome.

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Table 2: Effect of GH gene polymorphisms on some physiological parameters

Physiological parameters	BB	AB	AA
Glucose mg/dl	287.60±7.85a	271.08±3.90a	275.71±5.17a
Cholesterol mg/dl	133.22±9.56a	136.36±6.60a	145.70±6.58a
Triglyceride mg/dl	984.30±159.09a	1068.82±48.55a	1168.74±62.23a
V.L.D.L mg/dl	188.35±32.52a	216.34±10.26a	212.38±12.91a
Total protein gm/dl	6.78±0.33a	6.13±0.15a	6.60±0.19a

Means with the same superscripts in each row are insignificantly different

Effect of GH gene polymorphisms on weekly egg production

The results of table 3 showed no significant effect of various genotypes at GH gene (AA, AB, BB) of weekly egg production of hens from 1 to 14 and the significant effect was shown in the third week only (P<0.05) and the wild genotype AA had the highest mean 5.92 followed by the AB and BB genotypes (5.12 and 4.58) respectively. These results are agreed with the previous study of Makhsous *et al*.(2013) who refers to the positive relationship between the SNP of GH gene in native layer hens and these SNPs can be useful to improve the chicken breeding programs. Kuhnlein *et al*. (1997) refers that the genotypes have been characterized in the introns of cGH gene of White leghorn and it has been suggested that the alleles identified were linked to egg production phenotype.

Table 3: Effect of GH gene polymorphisms on weekly egg production

Egg production (egg)			
Weekly egg production	**BB**	**AB**	**AA**
1	3.72±0.37a	4.35±0.18a	4.60±0.26a
2	5.25±0.53a	4.63±0.21a	4.86±0.27a
3	4.58±0.46b	5.12±0.22ab	5.92±0.27a
4	5.61±0.45a	5.27±0.18a	5.48±0.25a
5	5.43±0.39a	5.30±0.20a	5.64±0.20a
6	5.25±0.52a	5.31±0.18a	5.95±0.22a
7	5.53±0.46a	5.56±0.19a	6.00±0.18a
8	4.77±0.47a	5.45±0.18a	5.57±0.24a
9	5.41±0.47a	5.85±0.15a	5.76±0.25a
10	5.58±0.42a	5.51±0.17a	5.79±0.21a
11	6.18±0.24a	5.80±0.14a	6.14±0.21a
12	5.50±0.43a	5.41±0.18a	5.67±0.19a
13	5.41±0.47a	5.48±0.18a	5.80±0.22a
14	5.10±0.43a	5.31±0.20a	5.49±0.24a
Total	82.75±1.58a	83.10±1.33a	84.30±2.07a

Means with the different superscripts within each row are significantly different (P<0.05)

Effect of GH gene polymorphisms on egg quality traits

The results of table 4 revealed that no significant effect of different genotypes of GH gene (AA, AB, BB) of egg quality traits. These results are disagreed with the study of Barkova et al.(2013) they refers to

positive association between egg shell thickness and genetic markers when study the expressed sequence ChEST985k21 as a regulating region in Rohde Island layers genome.

Table 4: Effect of GH gene polymorphisms on egg quality traits

Egg quality traits			
	BB	AB	AA
Shell thick	60.91±1.09a	61.28±0.53a	60.00±0.53a
Yolk diameter	57.28±0.53a	57.43±0.23a	56.89±0.22a
Albumin high	43.02±0.31a	43.12±0.14a	42.82±0.16a
Yolk high	20.31±0.33a	20.57±0.16a	20.24±0.19a
Width	9.68±0.31a	10.07±0.15a	9.87±0.19a
Long	41.30±0.61a	41.45±0.24a	40.51±0.31a
Egg weight	0.41±0.01a	0.41±0.008a	0.43±0.01a
Haugh unit	81.95±0.91a	82.18±0.37a	80.76±0.47a

Means with the same superscripts in each row are insignificantly different

Effect of GH gene polymorphisms on egg weight and live body weight

Table 5 observed no significant effect of GH gene polymorphisms on egg weight and live body weight in the different periods of this study. Mu'in and Lumatauw (2013) showed significant effect of the GH gene polymorphism in the fourth intron on the live body weight at 4 months of age and weight gain at 2-4 months of age of the native Indonesian chicken, also Anh *et al*. (2015) indicated the positive relationship between the various genotypes of the third intron of GH gene and live body at 4,6,8 and 10 weeks of age in layer hens. The differences between this study and the results above may be coming from the difference of the studied region and SNP of GH gene of previous studies in addition to the environmental effects on the layer hens which differs from country to other.

Table 5: Effect of GH gene polymorphisms on egg weight and live body weight

Age(weeks)	Egg weight (gm)		
	BB	AB	AA
Egg weight 1	65.43±1.10a	63.83±0.55a	65.20±0.75a
Egg weight 2	64.20±1.61a	62.02±1.16a	64.04±0.73a
Body weight 1	1.55±0.06a	1.47±0.02a	1.44±0.03a
Body weight 2	1.59±0.06a	1.55±0.02a	1.57±0.03a

Means with the same superscripts in each row are insignificantly different

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