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DETECTION OF C3678T SNP IN GROWTH HORMONE SECRETAGOGUE RECEPTOR (GHSR) GENE POLYMORPHISMS AND ITS EFFECT ON SOME OF PRODUCTION AND PHYSIOLOGICAL TRAITS OF IRAQI LOCAL CHICKEN

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Abstract

Ghrelin receptor or Growth Hormone Segretagogue Receptor (GHSR) gene is candidate gene associated with Productive Performance and physiological responses in chicken by modulating growth hormone which release from the pituitary by binding to its legend of ghrelin. Growth hormone secretagogue (GHS) effect on the feed intake and body weight, glucose and lipid metabolism, energy balance in birds and mammals. The aim of this study was to detect C3678T SNP in GHSR using PCR-RFLP and *Bsp119*l restriction enzyme and evaluate the association with some growth and physiological traits in Iraqi local chickens. 160 birds had been used in this study and reared in single cages. The result showed three genotypes Two alleles C and T and, wild type (CC) homozygous, heterozygous (CT), mutant (TT) homozygous. The size of the target fragment of GHSR gene was 598bp. The C allele frequency was 0.74, whereas T allele frequency was 0.26. The distribution percentage of GHSR gene polymorphisms for CC, CT and TT genotypes were 62%, 24% and 14%, respectively. The differences among percentages were significant ($P<0.01$). A significant ($P<0.05$) differences were showed between CC and TT genotypes at third and four weeks of age, the CC genotype was higher than TT and CT genotypes in egg production traits. There were no significant differences between CC, TT, CT genotypes in egg weight and body weight and physiological traits.

Key words : (GHSR) gene polymorphism, C3678T SNP**,** Iraqi local chicken.

Introduction

Somatotropic axis genes play an important role in chicken growth and developments such as growth, feed conversion, energy homeostasis, egg laying, carcass weight and body weight at different ages in domestic animals (Sewalem *et al.*, 2002; Nie *et al.*, 2005). The somatotropic axis or hypothalamus-pituitary growth axis consists of important component such as growth hormone (GH), leptin, insulin, ghrelin, GHSR, insulin-like growth factors (IGF –I and II), thyroid hormones and the receptors of these hormones (Nie *et al.,* 2005; Kadlec *et al*, 2011), Tanaka *et al*., (2003) discovered GHSR gene firstly known as Growth Hormone Segretagogue Receptor or ghrelin receptors, which consists of 4.1 kb, two exons divided by one intron responsible for production of 347 amino acids it is located on chromosome 9 (Tanaka *et al*., 2003; Nie *et al*., 2005). There are 37 SNPs registered in the cGHSR gene, containing 25 SNPs in intron, nine synonymous SNPs, two nonsynonymous SNPs, and one SNP in 3'UTR (Nie *et al.*, 2005). Ghrelin receptors are more complex and wide variety in non-mammals, than in mammals (Kaiya *et al*., 2014). The most of physiological functions of GHSR included: (1) the release of prolactin, cortisol, growth hormone and adrenocorticotropic hormone; (2) influences on lipid and glucose metabolism (Hosoda *et al*., 2006) (3); regulation of gastrointestinal secretion and motility (4) regulation of immune response; (5) play important roles in gastrointestinal balance (Smith *et al*, 2005; Murray *et al.*, 2005); (6) pancreatic function (Date *et al.*, 2002); (7) Sustainability of cell proliferation and survival (Mazzocchi *et al.*, 2004); (8) Protect the nervous system, blood vessels and heart cells (Baldanzi

et al., 2002; Jiang *et al.*, 2006). The diversity of functions of GHSR indicates the complexity of signals within GHSR cells. Therefore suggest several intracellular signaling methods to activate GHSR. In chicken previous studies have found a strong correlation between GHSR gene polymorphisms and growth traits, body consumption (Fang *et al.*, 2010; Niarami *et al.*, 2014). The purpose of this present study was to identify C3678T SNP in growth hormone secretagogue receptor (GHSR) genes and its association with some of production and physiological traits in Iraqi local chicken.

Materials and Methods

Five ml of blood was collected from the brachial vein of 150 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 (Noori *et al.*, 2019). The primers were supplied by Alpha DNA/Canada, as lyophilized powder of different picomols concentrations F-**5¹**TGGTTGAAAAGAGAGAATGCT**3¹** and R-**5¹** CCACACGTCTCCTTTTATATTC**3¹** gene bank (AB095994) according to (Anh Khoa *et al*, 2013)

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 ml. The amplification was carried out in the TECHNE (T-C 5000) thermal cycle and the reaction mixture was prepared according to the manufacturer's proposal (BIONEER, Korea) using 75-90 ng/ ml of DNA and 0.8 ml of primers and then complete the PCR reaction volume to 20 ml 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 94ºC for 4 minutes, 35 cycles of 94ºC for 30 seconds, 60ºC for 30 seconds, 72ºC for 30 seconds and a final elongation at 72 ºC for 5 minutes. PCR products (8m) were digested with 3 units of *Bsp119*l restriction enzyme at the 37ºC 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 weight and body weight 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.

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

Detection of enzyme Digestion using gel electrophoresis

The PCR products which underwent restriction digestion with *Bsp119*l enzyme (TT/GGAA) in order to detect SNP C3678T in exon 2 of the GHSR gene and it was able to cut this site of the wild genotype CC. The following fragments sizing patterns were observed by agarose gel electrophoresis.

1. Wild type CC: No cleavage of the whole 598 bp segment by *Bsp119*l. The results of present study
2. Heterozygous CT: *Bsp119*l was cut the sequence to show three fragments in agarose gel electrophoresis (598 bp, 426 bp and 172bp).
3. Homozygote TT: *Bsp119*l was cut the sequence to show two fragments on agarose gel electrophoresis (426bp, 172 bp) were similar with previous study of Anh Khoa *et al* (2013) on the Chines chicken.

TGGTTGAAAAGAGAGAATGCTATTTCATCTGAATCA
ACCTTTTTTTTTTTCTCCCTACTCACAGTTAA
CACACTTTCTTCTGCTGTTTTTTGTTTTAGTCGTG
GTGGTATTTGCTTTCATACTCTGCTGGTTGCCT
TTTCACGTAGGACGATATTTATTTTCCAAATCC

BSP119I

TT/CGAAGCCGGATCCTTGGAGATAGCAGTG
ATCAGCCAGTACTGCAACTTGGTGTCCTTTG
TCCTCTTCTACCTGAGCGCAGCCATCAACCCCA
TCCTCTACAACATCATGTCCAAGAAGTACCGC

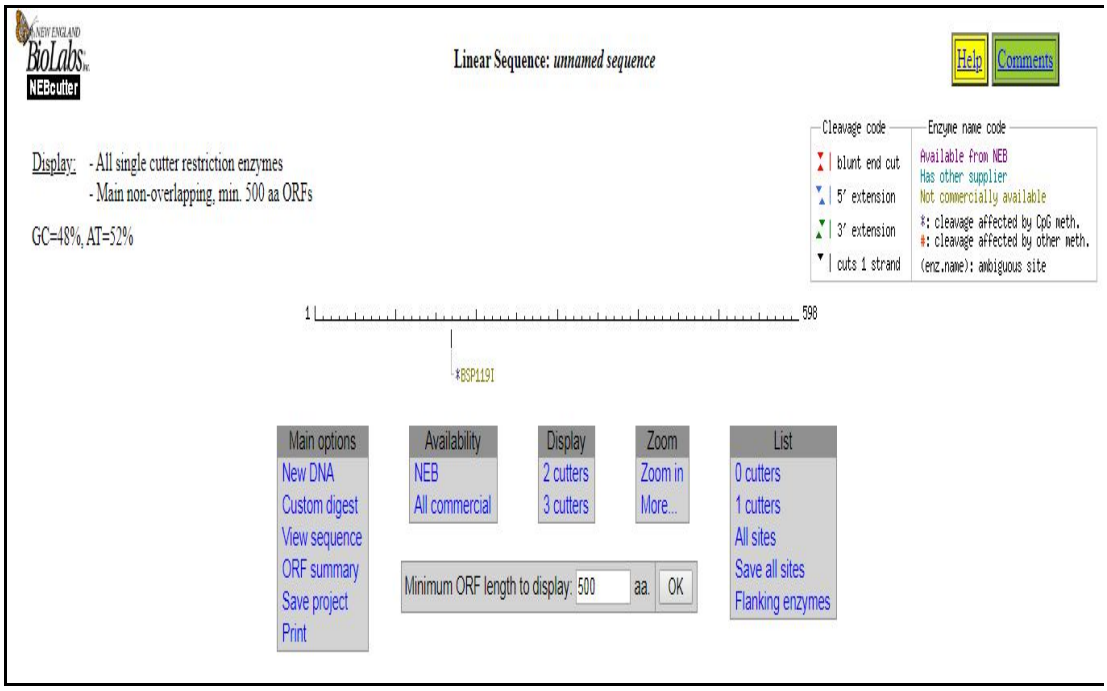

Fig. 2: PCR product of exon 2 of GHSR gene digested by *Bsp119I.*

GTTGCCGCATGCCGGCTCTTCGGACTCAAAGCC
CTGCCAAAGAAAAGACTCTCCAGCACCAAG
CAGGACAGCTCACGTGTATGGACAGAACCC
ACCGTCGCCACATGACACGGGGTCCAGTGCCAGA
GCATCACACACCGGTGTTACCGGAGAGCCATC
ACGGAGGGGACGTGGGGCAAGGCACAAATCGG
CAAGGAAAGCTCTACTTGTGTCGTCACCTGG
ATTTGCTGGGAGATGTCACGTAGGCAGTGAAAG
ATCTCTAAAAATTACCTACACCTTGAGAATA
TAAAAGGAGACGTGTGG

Distribution and allele frequency for GHSR gene polymorphisms of Iraqi local chicken

Table 1: Distribution percentage of different genotypes of Iraqi local chicken.

Genotype	No	(%)
CC	93	62.00
CT	36	24.00
TT	21	14.00
Total	150	100 %
Chi-square value ($\div^2$)	—	13.104**

Significant ** (P<0.01).

The results in Table 1 shows that the distribution of genotypes has reached the highest percentage of genotype CC (62%) compared with the genotype CT and TT genotypes with the results (24% and 14%), respectively With high level of significance (P <0.01). These results

Table 2: Allele frequency of GHSR gene of Iraqi local chicken.

Alleles	(%)
C	74
T	26
Total	100%

Significant ** (P<0.01).

are consistent with the results were obtained from previous studies of Anh Khoa *et al.,* (2013) when they detect the SNP C3678T on the GHSR gene in domestic breeds in China as well as in the commercial cobb 500 strain.

Allele frequency of GHSR gene of Iraqi local chicken

The results in table 2 indicated that the allele frequency of C allele was greater than 74% compared to the allele frequency of T allele 26% with a significant level of (P <0.01), were analyzed. Hardy Weinberg (HWE) equilibrium was used to estimate allele frequency (Solé *et al.,* 2006).

Effect of GHSR gene polymorphisms and its association with egg production of Iraqi local chicken

Results of table 3 showed there were no significant differences between CC CT and TT genotypes for the GHSR gene polymorphisms. Except in 3 and 4 week, results showed a significant differences (P<0.05) between CC and CT and TT genotypes were 5.80±0.39, 5.69±0.24 and 4.88±0.26 respectively, while CC genotype was superior than CT and TT genotypes and the results were

Table 3: Effect of growth hormone secretagogue receptor gene polymorphisms on egg production of Iraqi local chicken.

Egg production (egg)			Weeks of egg production
TT	CT	CC	
4.15±0.25[a]	4.43±0.19[a]	4.72±0.34[a]	1
4.93±0.26[a]	4.68±0.22[a]	4.73±0.42[a]	2
4.88±0.26[b]	5.69±0.24[ab]	5.80±0.39[a]	3
5.19±0.15[b]	5.35±0.23[b]	5.65±0.32[a]	4
5.29±0.22[a]	5.54±0.22[a]	5.51±0.26[a]	5
5.55±0.23[a]	5.50±0.21[a]	5.57±0.38[a]	6
5.79±0.19[a]	5.54±0.19[a]	5.94±0.33[a]	7
5.59±0.22[a]	5.36±0.22[a]	5.23±0.34[a]	8
5.91±0.18[a]	5.56±0.21[a]	5.91±0.32[a]	9
5.74±0.17[a]	5.45±0.16[a]	5.69±0.34[a]	10
5.78±0.18[a]	5.86±0.13[a]	6.17±0.26[a]	11
5.37±0.18[a]	5.53±0.22[a]	5.76±0.22[a]	12
5.48±0.16[a]	5.46±0.24[a]	6.05±0.23[a]	13
5.45±0.32[a]	5.33±0.22[a]	5.21±0.36[a]	14
82.73±1.74[a]	83.83±1.35[a]	84.66±2.56[a]	Total

Means with the same superscripts of each breed within each row are significantly different (P<0.05).

5.65±0.32, 5.35±0.23 and 5.19±0.15 respectively. Fang *et al*. (2010) reported also no effects of the T1857C SNS of GHSR gene Polymorphism In the performance of growth and production of the Xinghua chicken cross and Cross F2 White Recessive Rock.

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

Table 4: Effect of GHSR gene polymorphisms on egg weight and live body weight of Iraqi local chicken.

Age(weeks)	Egg weight (gm)		
	CC	CT	TT
Egg weight 1	64.48±0.65a	63.81±0.62a	63.68±1.17a
Egg weight 2	64.83±0.76a	62.24±1.16a	62.16±1.99a
Body weight 1	1.39±0.02a	1.48±0.03a	1.37±0.04a
Body weight 2	1.54±0.02a	1.58±0.03a	1.56±0.03a

Means with the same superscripts of each breed within each row are not significantly different.

Table5: Effect of GHSR gene polymorphisms on some Physiological traits of Iraqi local chicken.

Genotypes			Physiological traits
TT	CT	CC	
253.78±6.42[a]	272.12±6.67[a]	284.51±5.63[a]	Glucose(mg/dl)
136.64±9.41[a]	145.10±7.74[a]	152.69±9.03[a]	Cholesterol(mg/dl)
1027.62±69.89[a]	1194.32±53.72[a]	1163.33±68.57[a]	Triglyceride(mg/dl)
221.80±14.26[a]	255.27±10.98[a]	227.31±15.93[a]	V.L.D.L(mg/dl)
6.18±0.16[a]	6.43±0.19[a]	6.46±0.27[a]	Total protein (gm/dl)

(Table 4) showed no significant effect of GHSR gene polymorphisms on egg weight and live body weight in the different periods of study, this result not compatible with Kaiya *et al.*, (2013), it was found that Ghrelin regulates, differentiation and diffusion of cell through MAP kinase (MAPK) signals and GHS-R1a activation of growth hormone, eating and neutral activation through PLC / IP3 signals, the subsequent introduction of intracellular calcium into pituitary cells (Yin *et al.*, 2014). The differences between this study and the results above may be coming from the difference breeds of which studied the SNP of GHSR gene, in addition to the environmental effects on the layer hens which differs from country to other**.**

Physiological traits

The results in Table 5 pointed that there were no significant effects between CC, CT and AA genotypes on biochemical parameters (cholesterol, triglyceride, glucose, total protein, low density lipoprotein) for GHSR gene polymorphisms. The reason of non-significant effects between the various genotypes in the previous traits may be due to the many of genes with the growth hormone secretagogue receptor gene responsible effect on physiological traits and growth represented by increasing the number and size of body cells and the physiological characteristics represented by the different metabolic processes in the body of bird (d, Andre Hirwa *et al.*, 2010).

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