GHRELIN GENE POLYMORPHISMS AND EXPRESSION BOND WITH SOME GROWTH AND CARCASS TRAITS OF ROSS 308 BROILER CHICKENS

A.A. Joody * **D. H.Al-hassani*** **I. A. Abdul-Hassan****
Researcher **Prof.** **Prof.**
***Animal Production Dept., College of Agricultural Engineering Sciences, University of Baghdad**
****Institute for Genetic Engineering and Biotechnology, University of Baghdad**

ABSTRACT

This experiment was conducted to study the association of Ghrelin gene polymorphisms and expression levels with some productive and carcass traits of broiler chickens. Two hundred broiler chicks, one day old Ross308, were wing-tagged and reared under conventional conditions. Blood samples were collected individually of all birds to study of ghrelin gene polymorphisms by PCR-RFLP technique. Proventriculus was collected at 35 days of age from thirty birds of each groups sorted according to high, moderate and low growth rate to measure ghrelin gene expression by real-time RT-PCR. Result revealed that final body weight and weight gain were not significantly influenced by ghrelin gene , whereas body weight and weight gain of males and females with high ghrelin gene expression were significantly ($p<0.01$) higher than those of moderate and low ghrelin gene expression at 21 and 35 days of age. Ghrelin gene polymorphisms had no significant ($p>0.05$) influence on carcass traits and edible organ weights, while significant ($P < 0.01$) differences were existed among high, moderate and low ghrelin gene expression groups in carcass weight, breast, wing, neck and back relative weights. It is concluded that PCR- RFLP technique revealed no significant effect for ghrelin gene polymorphisms on productive and carcass traits, whereas ghrelin gene expression had significant effect on productive traits.

Keywords: polymorphisms, ghrelin, gene expression, body weight.

طرز جين الكرلين و مستويات تعبيره وعلاقتها مع بعض صفات النمو والذبيحه في فروج اللحم روز 308

احمد عبودي جودي* **ضياء حسن الحسني*** **اسماعيل عبد الرضا عبد الحسن****
باحث **استاذ** **استاذ**
***كلية علوم الهندسه الزراعية , قسم الإنتاج الحيوانية , جامعه بغداد , العراق .**
****معهد الهندسة الوراثية للدراسات العليا, جامعة بغداد**

المستخلص

أجريت هذه الدراسة لبحث العلاقة بين الطرز الوراثية لجين الكرلين و مستويات تعبيره مع بعض الصفات الإنتاجية لفروج اللحم. استخدم مائتان فرخ بعمر يوم واحد تم وزنها و ترقيمها بالجناح و ربيت تحت الظروف المثالية. جمعت عينات الدم من الأفراخ جميعها لدراسة الطرز الوراثية لجين الكرلين باستخدام تقنية تباين أطوال قطع التقييد RFLP المبنية على أساس التفاعل التضاعفي لسلسلة الدنا PCR . جمعت المعدة الغدية Proventriculus عند عمر 35 يوم من ثلاثون طير من كل مجموعة من الطيور التي تتميز بمعدلات النمو العالية, المتوسطة و الواطئة لتقدير التعبير الجيني للكرلين باستخدام تقنية real–time RT–PCR. أظهرت النتائج أن وزن الجسم النهائي و الزيادة الوزنية لم تتأثر معنويا بالطرز الوراثي لجين الكرلين لكنه أوزان الجسم و الزيادة الوزنية لذكور وإناث فروج اللحم التي تملك تعبير جيني عالي كانت واضحة الارتفاع ($p<0.01$) مقارنتا مع فروج اللحم الذي يمتلك تعبير جيني متوسط أو منخفض في اليوم 21 و 35 من العمر. عدم وجود تأثير معنوي ($p>0.05$) لطرز جين الكرلين في خصائص الذبيحة و الأوزان النسبية للأعضاء الصالحة للأكل لكن التأثير واضح ($P < 0.01$) بين مختلف مستويات التعبير الجيني للكرلين (المستوى العالي و المتوسط و المنخفض) في الأوزان النسبية للذبيحة, الصدر, الجناح, الرقبة والظهر. تقنية PCR– RFLP لم تظهر أي تأثير واضح لطرز جين الكرلين في أداء و خصائص الذبيحة لكن التعبير الجيني للكرلين اظهر تأثير واضح في أداء فروج اللحم.

الكلمات المفتاحية : الطرز الوراثي, الكرلين, التعبير الجيني و فروج اللحم

***Received:21/12/2018, Accepted:19/4/2019**

INTRODUCTION

During last three decades was found that gene polymorphisms can be adopted in genetic improvement and obtaining good production traits in organisms, so recently many candidate genes were used in improving physiological functions and productive performance in different poultry species (3, 4, 1). Endocrine system has involved in regulation growth of chicken by several pathways such as somatotropic and thyrotrophic axis, insulin like growth factor (IGF-1), insulin like growth factor binding protein (IGFBP) (6, 5, 14, 2). Somatotroph is one of the cell types that existed in the anterior pituitary gland, which are considered the major site of growth hormone (GH) secretion (24, 7) . Ghrelin is one of peptides that promotes GH secretion in birds through GH-Secretagogue Receptor (GHSR) existed in avian pituitary gland (31, 8, 11). Kojima et al., (16) defined ghrelin as the first endogenous ligand for the growth hormone Secretagogue receptor (GHS-R) which stimulates releasing GH. Ghrelin of most avian species is composed of 26 amino acids and sharing 54 % of amino acid sequence identity with human and rat ghrelin which generally consists of 28 amino acids in mature ghrelin peptide. After discovering ghrelin by (16) and its localization inside stomach by (30), studies launched giving much more attention on this hormone and its gene. Studies on ghrelin immuneopositive cells inside proventriculus showed that cells were not existed in the proventriculus on days 9 and 12 of incubation, whereas a few ghrelin immuneopositive cells were found in tubular part of proventriculus, which are 8.9 cells/mm^2 approximately. These cells are rounded, closed type and scattered in glandular epithelia not in the muscle layers or luminal epithelium of proventriculus (22). Kitazawa et al. (15) observed that ghrelin expression in proventriculus of white leghorn chickens goes down 60 % almost 3 days post-hatching then being stable for 100 days post-hatching, while most region of brain did not show changes in ghrelin expression which recorded low expression levels than that in proventriculus with exception in midbrain and medulla oblongata which revealed low expression after 3 days of hatching. Cerebral cortex and olfactory bulb showed higher ghrelin expression compared to pituitary, thalamus, medulla oblongata, cerebellum, hypothalamus and midbrain during 1 to 100 days of age. Yi et al., (35) found no significant differences in ghrelin gene expression in hypothalamus of two chicken lines selected for low and high body weight at 8 weeks of age being free access to feed and fasted for180 minutes. Sintubin et al.(29) noticed that broiler chickens with low feed residue had higher ghrelin mRNA in hypothalamus and this might be the reason of the differences in food consumption between the two lines. These results supported the previous findings of (33), who observed that ghrelin mRNA was clearly high at 1, 28 and 56 days of age in chicken lines selected for low body weight. Yamato et al., (34) studied the changes of ghrelin immuneopositive and mRNA expressing ghrelin cells during growth development of white leghorn chicks. The RT-PCR showed that ghrelin gene expression cells were found only in mucosal layer of proventriculus instead of myenteric plexus, at first day chick of age, whereas high levels of ghrelin expression cells in adult chicken compared to others tissues such as pylorus and duodenum, which have a low level (32). A number of studies have been conducted to evaluated ghrelin gene polymorphisms and expression on different avian species independently but very rear studied the association between polymorphisms and expression of ghrelin gene especially on commercial chicks so, the present study was designed to evaluate the association of polymorphisms and expression of ghrelin gene with some growth and carcass traits in Ross 308 broiler chickens.

MATERIALS AND METHODS

This study was conducted at poultry farm of AL-Aammeri hatchery, AL-Mahawel district, Babel governorate. Two hundred one day old broiler chicks (Ross308), 41- 44 g live body weight average were received, tagged individually by aluminum wing tags and housed in (2.5m×2.5m) floor pens. Feed and water were provided for birds ***ad Libitum*** during the experiment. Ration diets were formulated to meet the requirements mentioned by the National Research Council (

23) for broilers. Two types of diets were introduced to birds, starter diet containing 23.6% crude protein and 3026 kcal ME/kg diet during first three weeks of age and finisher diet containing 21% crude protein, and 3197 kcal ME/kg diet during last two weeks of age. All birds were vaccinated against Newcastle and Gumboro diseases. Individual live body weight and Body weight gain of birds were weekly recorded, then the means of each sex were calculated during the fattening period . Blood samples were collected individually from all birds. One milliliter of each blood samples were collected from wing vein into tubes coated with EDTA and stored at -18 ^{0}C until analysis. At the end of experiment, thirty broiler chickens (15 males and 15 females) of each from high, moderate and low growth rates were selected and slaughtered, de-feathered, eviscerated and the carcass was cut into major and minor parts to measure absolute and percentage weights. Total DNA from 200 µl of whole frozen blood was extracted using the gSYNC™ DNA Extraction Kit (Geneaid USA) as described in instruction manual. The concentration of DNA samples and the purity was estimated by nanodrop. The PCR amplification for ghrelin gene was carried out using Accu Power PCR PreMix KIT (Bioneer, Korea). The sequence of primers that used to characteristic ghrelin gene (GenBank: JN578262.1) were, Forward; 5'AAGGACACGTGGAAACTGCCAGC3', Reverse;
5'AAGCAGCCTGAGGTGACTGCAA3' (25). The amplification program were as follows: Initial denaturation at 94℃ for 3 minutes , followed by 35 cycles of denaturation at 94℃ fo 30 seconds, annealing at 60℃ for 45 seconds, and extension at 72℃ for 1 minute , with final extension at 72℃ for 5 minutes, then hold at 4℃ . The product was digested by ***Hinf I*** restriction enzyme. At 35 days of age, thirty chickens of each from high, moderate and low growth rates were selected and slaughtered to collect proventriculus tissue for evaluation ghrelin gene expression. Proventriculus samples were frozen in liquid nitrogen until analysis. Total RNA was extracted from proventriculus tissue using Total RNA Mini Ki (Geneaid USA). Conversion of mRNA into double-stranded cDNA carried out by using AccuPower® RocketScript™ RT PreMix kit (Bioneer - Korea). Complementary DNA of ghrelin was amplified with the following primers: F: CCT TGG GAC AGA AAC TGC TC and R : CAC CAA TTT CAA AAG GAA CG with using chicken 18S ribosomal RNA F :CGCGTG CAT TTA TCA GAC CA , R :ACC CGT GGT CAC CAT GGT A (Gene Bank accession no AF173612) as an internal control in qPCR. AccuPower® GreenStarTM qPCR PreMix was used to mRNA expression quantified using the Exicycler™ 96 (Exicycler™ 96 Real-Time Quantitative Thermal Block, Bioneer Co.) apparatus according to following protocol: one cycle of Pre Denaturation 95 °C, 1-5 min, 40-45 cycles of Denaturation 95 °C, 5-20 sec, Annealing/Extension at 55-60 °C for 40-45 sec, Detection(Scan) then one cycle of Melting. After completion reaction, all data were obtained using Exicycler 96 Real-time Quantitative Thermal Block (Bioneer Co.). The CT for each sample was determined by Exicycler 96 detection software (version 1.2, Bioneer) and quantitative was calculated by 2-ΔΔCt method (18). Data were subjected to analysis of variance (26) and significant means were calculated by Duncan's multiple range test (9).

RESULTS AND DISCUSSION

Tables **1, 2** and **3** illustrate that ghrelin gene polymorphisms of Ross 308 broiler chickens had no significant effect on live body weight, body weight gain and all carcass traits at 1, 7, 14, 21, 28 and 35 days of age**. Table 4** revealed strong bond between Ghrelin gene expression level and live body weight of broiler chickens, so the same table exhibited significant differences between the three levels of ghrelin gene expression and live body weight of broiler chickens in both sexes at 21 and 35 days of age, which was in favor of higher expression levels. Ghrelin gene expression level had a significant influence on each of carcass weight in males and females and their average (table **5**), breast and wing weights (table **6**), average relative weight of gizzard and heart (table **7**). On the other hand ghrelin gene expression had no significant effect on each of dressing percentage of males and females and their average (table **5**), thigh

and drumstick weights (table **6**), and relative weight of liver (table **7**). Our results correspond with results obtained by (28), who studied ghrelin polymorphisms in Ross and Cobb broiler chickens, and they found no significant association between body weight, and carcass characteristics with ghrelin gene polymorphisms. Despite, Fang et al., (10) found a positive association between 8 bp indel polymorphism in ghrelin gene with body weight and some carcass characteristics. Three genotypes of ghrelin gene were observed among them, BB genotypes had a positive association with body development and growth traits, while the association of single nucleotide polymorphisms (SNPs) of ghrelin gene studied in Chaohu duck by PCR-SSCP analysis (13). Also, Li et al.,(17) detected a positive correlation between ghrelin gene polymorphisms (TT, CC and TC genotypes) and growth traits in Chinese indigenous and commercial chickens. Shahryar and Lotfi (27) noticed that injection geese with ghrelin led to significant increase in breast weight percentage as compared to control group, whereas no significant effect was found on carcass yield, thigh, and liver weights. Previous results confirmed by (19) who concluded that the positive effect of ***in ovo*** ghrelin injection on carcass characteristics, breast, thigh, gizzard, liver, heart and intestine, except the carcass yield. On the other hand, Lotfi and Shahryar (20) did not find significant effect on liver, heart and breast weight percentage, with slight increase in gizzard weight percentage was found following exogenous ghrelin ***in ovo*** injection on newly hatched chickens. Positive effect of ghrelin gene expression may be because of the significant physiological role of ghrelin played on some biochemical pathways strongly related with growth and feed intake of broiler chickens (12). From this study we can conclude that ghrelin gene expression is a precise and effective technique to use in selection programs of broiler chickens for the rapid growth.

Table 1: Effect of Ghrelin gene polymorphisms on body weight (g) of Ross 308 broiler chickens (Means ± SE)

Age (Days)	Genotypes of Ghrelin gene		p value
	GG	LL	
1	40 ± 0.18	40 ± 1.67	NS
7	149 ± 1.27	146 ± 11.89	NS
14	420 ± 3.06	420 ± 33.22	NS
21	777 ± 7.16	781 ± 75.06	NS
28	1375 ± 13.54	1435 ± 154.02	NS
35	1888 ± 60.93	1984 ± 208.73	NS

NS: No Significant difference

Table 2 Effect of ghrelin gene polymorphisms on body weight gain (g) of Ross 308 broiler chickens (Means ± SE).

Age (Days)	Genotypes of Ghrelin gene		P value
	GG	LL	
1 – 7	109 ± 1.16	105 ± 10.40	NS
8 – 14	272 ± 2.31	274 ± 21.36	NS
15 – 21	357 ± 6.02	361 ± 49.09	NS
22 – 28	598 ± 11.47	654 ± 79.02	NS
29 – 35	513 ± 60.91	550 ± 81.39	NS
1 – 35	1848 ± 60.91	1944 ± 207.40	NS

NS: No Significant difference

Table 3. Effect of ghrelin gene polymorphisms on Live body weight, Carcass weight, dressing and internal organs weight percentages in Ross 308 broiler chickens at 35 days of age (Means ± SE).

Characters	Genotypes of Ghrelin gene		P value
	GG	LL	
Live body weight (g)	1800.85 ± 33.71	1984.33 ± 208.73	NS
Carcass weight (g)	1441.17 ± 28.27	1588 ± 159.12	NS
Dressing (%)	79.98 ± 0.41	80.15 ± 0.53	NS
Thigh (%)	25.50 ± 0.27	25.05 ± 0.58	NS
Drumstick (%)	10.64 ± 0.24	10.71 ± 0.73	NS
Breast (%)	32.98 ± 0.32	33.59 ± 1.44	NS
Wing (%)	8.87 ± 0.12	8.81 ± 0.57	NS
Neck and back (%)	13.28 ± 0.21	13.37 ± 1.67	NS
Liver (%)	3.67 ± 0.08	3.45 ± 0.29	NS
Gizzard (%)	3.26 ± 0.08	3.58 ± 0.15	NS
Heart (%)	0.80 ± 0.24	0.79 ± 0.13	NS
Pancreas (%)	0.19 ± 0.01	0.19 ± 0.04	NS

NS: No Significant difference

Table 4. Effect of different levels of ghrelin gene expression on body weight (g) in male and female Ross 308 broiler chickens at 21 and 35 days of age (Means ± SE).-

Age (Days)	Sex	Ghrelin Gene Expression			p value
		High	Moderate	Low	
21 days	M	851.22 ± 15.09^{a}	781.00 ± 18.19^{b}	740.44 ± 47.30^{b}	**
	F	835.17 ± 15.22^{a}	782.70 ± 16.47^{ab}	686.74 ± 21.87^{b}	**
	Average	847.21 ±11.87^{a}	781.90 ± 12.06^{b}	704.00 ± 21.28^{c}	**
35 days	M	2207.33 ± 37.83^{a}	1864.72 ± 37.05^{b}	1555.89 ± 122.62^{c}	**
	F	2037.00 ± 29.22^{a}	1779.02 ± 22.90^{b}	1380.26 ± 52.85^{c}	**
	Average	2164.75 ± 32.81^{a}	1819.61 ± 22.14^{b}	1436.71 ± 54.26^{c}	**

In this and following tables gene expression values were expressed as delta ghrelin / delta 18S r RNA ratios. These ratios were ranged more than 0.93, 0.65-0.75 and less than 0.59 for high, moderate and low levels, respectively. * and ** means a significant differences at (p≤0.05) and (p≤0.01) levels, respectively

Table 5. Effect of different levels of ghrelin gene expression on Carcass weight and dressing percentage in males and females of Ross *308* broiler chickens at 35 days of age (Means ± SE).

Characters	Sex	Ghrelin Gene Expression			p value
		High	Moderate	Low	
Carcass weight (g)	M	1785.89 ± 32.54^{a}	1484.33 ± 25.92^{b}	1238.44 ± 97.43^{c}	**
	F	1630.83 ± 51.09^{a}	1425.35 ± 18.74^{b}	1149.79 ± 34.06^{c}	**
	Average	1747.13 ± 30.41^{a}	1453.29 ± 16.24^{b}	1178.29 ± 38.63^{c}	**
Dressing percentage (%)	M	80.94 ± 0.73^{a}	79.70 ± 0.70^{a}	79.71 ± 1.98^{a}	NS
	F	80.00 ± 1.67^{a}	80.22 ± 0.89^{a}	79.24 ± 0.89^{a}	NS
	Average	80.71 ± 0.67^{a}	79.97 ± 0.57^{a}	79.40 ± 0.86^{a}	NS

*** = Significant difference at (p≤0.05) level ** = Significant difference at (p≤0.01) level**

NS: No Significant difference

Table 6. Effect of different levels of ghrelin gene expression on thigh, drumstick, breast, wing, neck and back weight percentage in males and females of Ross 308 broiler chickens at 35 days of age (Means ± SE).

Characters %	Sex	Ghrelin Gene Expression			P value
		High	Moderate	Low	
Thigh weight	M	25.21 ± 0.75 [a]	26.20 ± 0.44 [a]	24.69 ± 0.87 [a]	NS
	F	25.21 ± 0.82 [a]	25.21 ± 0.65 [a]	25.80 ± 0.47 [a]	NS
	Average	25.21 ± 0.59 [a]	25.68 ± 0.40 [a]	25.44 ± 0.43 [a]	NS
Drumstick weight	M	11.60 ± 0.74 [a]	10.26 ± 0.25 [a]	10.71 ± 0.41 [a]	NS
	F	10.04 ± 0.64 [a]	10.31 ± 0.68 [a]	10.62 ± 0.34 [a]	NS
	Average	11.21 ± 0.59 [a]	10.29 ± 0.37 [a]	10.65 ± 0.26 [a]	NS
Breast weight	M	34.59 ± 0.82 [a]	33.21 ± 0.72 [a]	31.03 ± 0.70 [b]	**
	F	34.41 ± 0.66 [a]	33.48 ± 0.49 [a]	31.30 ± 0.55 [b]	**
	Average	34.54 ± 0.63 [a]	33.35 ± 0.42 [a]	31.21 ± 0.43 [b]	**
Wing weight	M	8.09 ± 0.15 [b]	9.05 ± 0.21 [a]	9.44 ± 0.42 [a]	**
	F	7.94 ± 0.21 [b]	9.02 ± 0.20 [a]	9.30 ±0.32 [a]	*
	Average	8.05 ± 0.12 [a]	9.03 ± 0.14 [a]	9.34 ± 0.25 [b]	**
Neck and back weight	M	12.97 ± 0.53 [b]	12.52 ± 0.47 [b]	14.94 ± 0.44 [a]	**
	F	13.52 ± 0.62 [a]	13.16 ± 0.35 [a]	13.61 ± 0.52 [a]	NS
	Average	13.10 ± 0.42 [a]	12.86 ± 0.29 [ab]	14.04 ± 0.40 [b]	*

*** = Significant difference at (p≤0.05) level ** = Significant difference at (p≤0.01) level ,**
NS: No Significant difference

Table 7. Effect of different levels of ghrelin gene expression on giblet weight percentage in males and females of Ross 308 broiler chickens at 35 days of age (Means ± SE).

Characters	Sex	Ghrelin Gene Expression			p value
		High	Moderate	Low	
Liver	M	3.30 ± 0.09[a]	3.71 ± 0.09[a]	4.02 ± 0.21[a]	NS
	F	3.37 ± 0.10[a]	3.51 ± 0.18[a]	4.05 ± 0.23[a]	NS
	Average	3.14 ± 0.07[a]	3.61 ± 0.10[b]	4.04 ± 0.17[b]	**
Gizzard	M	2.92 ± 0.15[a]	3.40 ± 0.23[a]	3.25 ± 0.25[a]	NS
	F	3.04 ± 0.10[a]	3.51 ± 0.19[a]	3.32 ± 0.13[a]	NS
	Average	2.95 ± 0.12[a]	3.46 ± 0.15[ab]	3.30 ± 0.12[b]	*
Heart	M	0.73 ± 0.03[b]	0.86 ± 0.06[ab]	0.93 ± 0.10[a]	*
	F	0.72 ± 0.04[a]	0.71 ± 0.04[a]	0.88 ± 0.06[a]	NS
	Average	0.73 ± 0.02[a]	0.78 ± 0.04[ab]	0.90 ± 0.05[b]	*

*** = Significant difference at (p≤0.05) level ** = Significant difference at (p≤0.01) level**
NS: No Significant difference

REFREANCE

1. Abdulwahid H. S., D.H.Al-Hassani and W.M. Razuki, 2019. Association of very low density lipoprotein receptor (VLDLR) gene polymorphisms with egg production traits in Iraqi Local Chickens. The Iraqi Journal of Agricultural Sciences. 50 (2):727 - 733.

2. Al-Hassani, A.S., I. A. Abdul-Hassan and D. H. Al-Hassani,2018. Some Biochemical traits in broiler chicken as affected by insulin-like growth factor-1(IGF-1) gene polymorphisms. Journal of Entomology and Zoology Studies; 6(2): 864-868.

3. Al-Hassani, A.S. D.H. Al-Hassani and I.A. Abdul-Hassan,2015. Association of Insulin-Like Growth Factor-1 Gene Polymorphism at 279 Position of the 5'UTR Region with Body Weight Traits in Broiler Chicken. Asian Journal of Poultry Science 9 (4): 213-222.

4. Al-khatib, B. G. and D. H. Al-Hassani, 2016. Effect of G1705A SNP in Growth Hormone Gene on the Productive and Physiological Performance in Broiler chicken. Iraqi Journal of Biotechnology. Vol. 15, No.1, 33-45.

5. Armstrong D. G. and C. O. Hogg, 1994. Type-I insulin like growth factor receptor gene expression in the chick: Developmental changes and the effect of selection for increased growth on the amount of receptor m

RNA. Journal of Molecular Endocrinology. 12:3-12.

6.Ballard F J, R J. Johnson, P. C. Owen, G. L, Francis, F. M, Upton, J. P, McMurtry, and J. C. Wallace, 1990. Chicken insulin-like growth factor-I: Amino acid sequence, radioimmunoassay, and plasma levels between strains and during growth. General and Comparative Endocrinology. 79:459-468.

7.Dean C E, Piper and M, T. E, Porter 1997. Differential responsiveness of somatotrophs to growth hormone-releasing hormone and thyrotropin-releasing hormone during chicken embryonic development. Molecular and Cellular Endocrinology 132:33-41.

8.Dieguez C, and F. F, Casanueva 2000. Ghrelin: a step forward in the understanding of somatotroph cell function and growth regulation. European Journal of Endocrinology 142 413–417.

9.Duncan D. B, 1955. Multiple Range and Multiple Ftests. Biometrics, 11: 1-42.

10.Fang M, Q, Nie C Luo, D, Zhang and X, Zhang 2007. An 8bp indel in exon 1 of Ghrelin gene associated with chicken growth. Domestic Animal Endocrinology. 32; 216-225.

11.Gaylinn B D, S. Harvey, and A. A, Toogood 2000. Cloning of growth hormone-releasing hormone receptor and growth hormone secretagogue receptor from chicken. In: Dawson A, editor. International Symposium on Avian Endocrinology, Varanasi, India; Abstract 6.04.

12.Jody, A. A., A. A. Abdulhassan and D. H. Al-hassani 2018. Association of ghrelin gene polymorphisms and expression levels with some biochemical traits in broiler chickens. The Iraqi Journal of Agricultural sciences. 49, (5): 870-878.

13.Jun-ying L, 2009. SNP Detection on the Polymorphism of Ghrelin Gene of Chaohu Duck and Association Analysis on its Polymorphism and Body Measurement Traits. Journal of Anhui Agricultural Sciences 37(10): 4443 – 4445.

14.Kim J W and Y. R . Boisclair, 2008. Growth hormone signaling in the regulation of acid labile subunit. Asian Australasian Journal of Animal Sciences. 21:754-768.

15.Kitazawa T, T, Hiraga H, Teraoka N, Yaosaka and H, Kaiya 2015. Correlation of ghrelin concentration and ghrelin, ghrelin-O-acetyltransferase (GOAT) and growth hormone secretagogue receptor 1a mRNAs expression in the proventriculus and brain of the growing chicken. Peptides 63:134–140.

16.Kojima M, H, Hosoda Y, Date M, Nakazato H, Matsuo and K, Kangawa 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402 656–660.

17.Li C C, K, Li, J, Li, D. Mo L, R F, Xu, G. H. Chen1, Y Z, Qiangba, S. L. Ji, X. H. Tang, B, Fan M. J, Zhu T. A. Xiong, X, and B. Guan Liu, 2006. Polymorphism of Ghrelin Gene in Twelve Chinese Indigenous Chicken Breeds and Its Relationship with Chicken Growth Traits. Asian Australasian Journal of Animal Sciences. 19 : 153-159.

18.Livak K J, Schmittgen and T. D, 2001. Analysis of relative gene expression data using realtime quantitative PCR and the $2^{-\Delta\Delta Ct}$ Method. *Methods* 25(4): 402–408.

19.Lotfi A, A. H. Shahryar , and H Kaiya , 2013. Effect of in ovo ghrelin administration on hatching results and post-hatching performance of broiler chickens, Livestock Science, 154, 158–164.

20.Lotfi A, and A . H, Shahryar 2012. Effect of in ovo administration of exogenous ghrelin on weight of intestinal organs in newly hatched chicks. Trends in biotechnology research 2: 20-22.

21. Ma J., Yu Y., J Xue., C. Ou, H. Mo., and X., Liu 2015. Tissue distribution and developmental changes of ghrelin and GOAT expression in broiler chickens during embryogenesis. General and comparative Endocrinology 213: 130-135.

22. National Research Council, N. R. C. 1994. National Academy of Science. Nutrient Requirement of Poultry 9th ed. Washington, USA.

23.. Porter T E, G . C. Couger, C. E, Dean and B. M. Hargis, 1995. Ontogeny of growth hormone (GH)-secreting cells during chicken embryonic development: initial somatotrophs are responsive to GH-releasing hormone. Endocrinology 136:1850- 1856.

24.. Sadegh M E, and C S, Nagaraja 2012. DNA Polymorphism studies on ghrelin gene In chicken. Indiana Journal of Applied Research 1:188-189.

25.. SAS, 2000. Propriety Software Release 8.1. Statistical Analysis Systems Institute, INC., Cary, North Carolina, USA.

26.. Shahryar A H, and A. Lotfi, 2015. The effect of peripheral administration of ghrelin on the performance of growing geese, Archives Animal Breeding., 58, 211-216.

27.. Sharifinejad H, M. B. M, Torbati and H, Farhangfar 2015. Study of Polymorphism in Exon 1 and Exon 2 Ghrelin Gene and its Association with Growth Traits in Broiler Chickens. Genetics in the 3rd Millennium,134046: 4052.

28. Sintubin P, E, Sintubin A,Greene A,Collin T,Bordas S, Zerjal J, Tesseraud S, Buyse and B, Dridi 2014. Expression profile of hypothalamic neuropeptides in chicken lines selected for high or low residual feed intake. Neuropeptides 48: 213–220.

29.. Tomasetto C, S. M, Karam S, Ribieras R, Masson O . Lefebvre, A. Staub, G .Alexander, M. P, Chenard and M.C. Rio , 2000. Identification and characterization of a novel gastric peptide hormone: the motilin-related peptide. Gastroenterology 119 395–405.

30. Toogood A A, S. Harvey, M. O. Thomer, and B. D, Gaylinn 1999. Cloning the chicken growth hormone Secretagogue receptor. In: Proceedings of the 81st Annual Meeting of the Endocrine Society 2–479. p. 382.

31. Wada R, I .Sakata, H. Kaiya, K, Nakamura Y,Hayashi K, Kangawa and T. Sakai, 2003. Existence of ghrelin-immunopositive and -expressing cells in the proventriculus of the hatching and adult chicken. Regulatory Peptides 111:123–128.

32. Xu P, P. B. Siegel, and D. M, Denbow 2011. Genetic selection for body weight in chickens has altered responses of the brain's AMPK system to food intake regulation effect of ghrelin, but not obestatin. Behavioural Brain Research. 221:216–226.

33. Yamato M, I, Sakata H Wada Kaiya,and X. Sakai 2005. Exogenous administration of octanoic acid accelerates octanoylated ghrelin production in the proventriculus of neonatal chicks. Biochemical Biophysical Research Communications, 333, 583–589.

34. Yi J, E. R, Gilbert, P. B. Siegel, and M. A, Cline 2015. Fed and fasted chicks from lines divergently selected for low or high body weight have differential hypothalamic appetite-associated factor mRNA expression profiles Behavioural Brain Research 286: 58–63.