ASSOCIATIONS OF VERY LOW-DENSITY LIPOPROTEIN RECEPTOR (VLDLR) GENE POLYMORPHISMS WITH EGG PRODUCTION TRAITS IN IRAQI LOCAL BROWN CHICKENS

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ABSTRACT

Very low-density lipoprotein receptor gene (VLDLR) is a part of the low-density lipoprotein receptor (LDLR) family. VLDLR plays important roles in transport egg yolk precursor to the oocyte. This work was conducted to study the effect of VLDLR polymorphism on the productive performance of local Iraqi chickens. In this study, the VLDLR gene was studied using blood samples of 173 pedigreed hens aged 47 weeks representing the third generation selected for high egg production. Genotypic and alleles frequencies in addition to the association between genotypes and egg production, egg weight and egg mass from onset eggs laid to the 80 weeks of age were investigated. The results showed that the genotypic and allelic frequencies of the VLDLR in 173 chickens did not agree with Hardy-Weinberg equilibrium (P<0.0307). The genotypic frequencies were 94.7 and 5.3% for both GG and GT genotypes respectively while, the allele frequency of genes G and T were 97.4 and 2.6%, respectively. The results showed an increase (P<0.0001) in egg production, egg weight and egg of GG genotypes compared to GT genotypes. This finding supports the previous results of VLDLR as a compromising gene. The current results approved that the VLDLR gene can be used as a candidate gene for modulating and improving egg production traits in Iraqi local chickens.

Keywords: Candidate gene, Egg traits, Local chickens, SNP, VLDLR
***Part of M.Sc. thesis for the 1st author**

دراسة العلاقة بين جين مستقبل البروتينات الدهنية واطئة الكثافة جداVLDLR وصفات انتاج البيض في الدجاج المحلي البني العراقي

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المستخلص

يعد جين مستقبل البروتين الدهني منخفض الكثافة (VLDLR) جزأً من عائلة مستقبل البروتين الدهني منخفض الكثافة (LDLR) لدوره المهم في نقل مكونات صفار البيض إلى البويضة. اجري هذا البحث لدراسة تأثير طرز جين VLDLR في الأداء الإنتاجي للدجاج العراقي المحلي. في هذه الدراسة ، تمت دراسة الجين VLDLR باستخدام عينات دم من 173 دجاجة محلية منسبة بعمر 47 أسبوعًا تمثل دجاج الجيل الثالث المنتخب لزيادة إنتاج البيض. تم فحص التكرارات الوراثية والاليلية والارتباط بين الأنماط الجينية وإنتاج البيض ، ووزن البيض وكتلة البيض من عمر النضج الجنسي لغاية عمر 80 أسبوعًا . أظهرت النتائج أن التكرار الوراثي والاليلي لـ VLDLR في 173 دجاجة لم يتفق مع توازن هاردي–فاينبرغ (P <0.0307). إذ كانت التكرارات الوراثية 94.7 و 5.3٪ لكل من التراكيب الوراثية GG و GT على التوالي، بينما كان التكرار الأليلي G و T بمقدار 97.4 و 2.6٪ على التوالي. وأظهرت النتائج زيادة (P <0.0001 في إنتاج البيض ووزن البيض وكتلة البيض لمجموعات الطيور التي تتبع الأنماط الوراثية GG مقارنة بتلك التي تتبع الأنماط الجينية GT. هذه النتيجة تدعم النتائج السابقة التي بينت امكانية اعتماد لـ VLDLR كجين مهم في انتاج البيض. تقترح نتائج الدراسة الحالية امكانية استخدام جين VLDLR كأحد الجينات المرشحة لتعديل وتحسين صفات إنتاج البيض في الدجاج المحلي البني العراقي.

الكلمات المفتاحية: الجينات المرشحة، صفات البيض، الدجاج المحلي، اشكال النيوكلوتيدات المفردة، مستقبل البروتينات الدهنية منخفضة الكثافة جداً

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INTRODUCTION

Over past five decades ago, a breeding program in poultry successes to produce breeds of chickens specialized in egg and meat production that exceed the production of their wild ancestries. The high production of those breeds has been achieved through selection and breeding of superior birds which results in increasing the frequency of favorable alleles in the next generation. Many technologies such as reciprocal recurrent selection, selection index, and BLUP were introduced in layer breeding and achieved great success in improving the performance of birds (17). In Iraq and in other developing countries, the accuracy of determining the superior birds through pedigree is still difficult to be done due to impairment in investment in livestock improvement through breeding. So, discovering genetic markers that associate with egg performance is crucial for layer breeding. One of these genetic markers is a very low-density lipoprotein receptor (VLDLR). The VLDLR is a member of the low-density lipoprotein receptor family (15), also named vitellogenesis receptor (OVR) or vitellogenin receptor (VTGR), mediated the absorption of yolk proteins from plasma very low-density lipoproteins and vitellogenin (20), It also acts as a part of triglycerides and cholesterol metabolism (6), as well as in many cellular processes of cell proliferation, migration, and differentiation (11). VLDLR in chicken (5; 19) and other oviparous species (10) involves in reproduction through its role in the development of oocytes and yolk lipoprotein deposition. Meng *et al*. (13) found the level of egg production in force molted chickens dependent on the level of VLDLR expression in the ovary and Han *et al*. (10) showed that VLDLR mRNA expression has a pivotal role in reproduction. Many of studies have shown the importance of very low density lipoprotein (VLDLR) gene as a candidate gene to modulate egg production in chickens (21), zebra finch (10), ducks (20) and quail (24). The Iraqi local chicken is one of the most important genotypes that must be preserved, which represents gene bank of adapted and resistant traits to Iraqi harsh environment (2 ;12: 13). Performance of local chicken's ecotype remains poor and still produces fewer eggs than standard commercial breeds. Therefore improve their performance through traditional selection and the genetic marker is an issue. Marker-assisted selection has become important tools for improving productive and reproductive performance in breeding programs and due to limited studies on using genetic markers toward improving production traits of local chickens. An experiment was conducted to assess the association between VLDLR gene and egg production traits in local Iraqi Brown chickens selected for high egg production for three successive generations**.**

MATERIALS AND METHODS

Site of study

This work was carried out at the farm of Poultry Research Station of Office of Agricultural Research, Abu Ghraib - Baghdad from the period from 1/October/2017 until 16/May/2018. Laboratory work was carried out in the laboratories of the Institute of Genetic Engineering and biotechnology Institute for Postgraduate Studies, the University of Baghdad for isolation of DNA and determines the genotypes of the VLDLR gene.

Birds and data collection

One hundred and seventy-three Iraqi local Brown Chickens were used, which represented the third generation of parents flocks selected for high egg production. Hens were kept in an individual cage and their egg production was recorded daily from onset egg to 80 weeks of age. Egg number, egg weight and egg mass for each was recorded daily and summarized in for the period, 20 to 40, 40 to 60, 60 to 80 and 20 to 80 weeks of age. All hens from 11 sire families in one hatch and reared in same environmental conditions. Ad libitum feed was presented as mash form. Hens were provided lighting regime of 16L: 8D (Light: Dark). Water supply was a freely.

Blood samples and DNA isolation

Blood samples were drawn from wing vein of 47 weeks old hen by collecting 3 ml of each inside test tubes containing EDTA anticoagulant produced by the Jordanian company AFCO. The samples were placed inside the refrigerated box and immediately stored at -20 º C until laboratory analysis(1). Chicken genomic DNA was extracted from

blood by a phenol/chloroform method. The primers and restriction enzymes used for polymerase chain reaction (PCR) forward: 5'-TCTATGGTGCCAACAAAT-3'. reverse: 5'-CATCTCAGACCGTCCTCC -3' and restriction enzymes (*Eco57I*). SNP genotypes were detected by PCR-restriction fragment length polymorphism (PCR-RFLP). The PCR was performed by mixing 3µl of genomic DNA with 1µl of each of forward and reverse primer, 12.5 µl of Go Taq® Green Master Mix, 7.5 µl of nuclease-free water in 25 µl total volumes, and was run on Eppendorf gradient tubes according to the following procedure: denaturation 94º C for 5 min for one cycle and 30 cycles for 30 seconds, annealing of 55º C for 45 second, primary extension 72 ºC for 30 second and final extension 72º C for 5 min for one cycle.

Statistical analysis

The genotypic frequencies were calculated and Hardy-Weinberg equilibrium was tested through using Chi-square test ($\chi 2$) of the FREQ procedure. One-way analysis of variance by the general linear model (GLM) procedures was used to examine the association between very low-density lipoprotein receptor with egg production, egg weight and egg mass in Iraqi local brown chickens. Values are considered significant at $P<0.05$ and are presented as a mean± standard error. All analysis was conducted by using the SAS software package (18).

RESULTS AND DISCUSSIONS

DNA extraction

DNA extraction was successful performed. Then samples of extracted DNA were electrophoretic on Agarose gel to visualize the DNA as shown from Figure1.

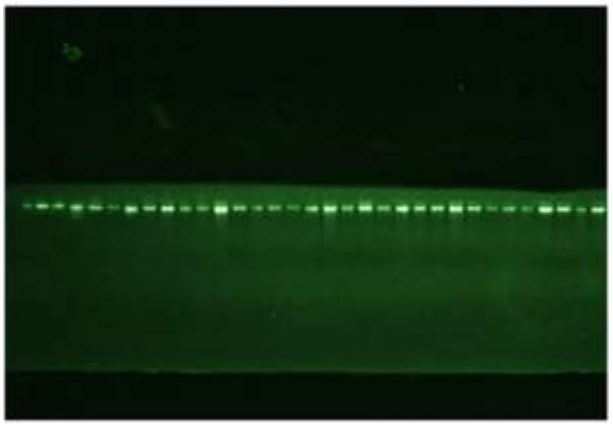

Figure 1. DNA extraction product on agarose gel(1%)

Gene Extraction (VLDLR)

The VLDLR gene fragment was amplificated using the PCR technique, samples of DNA product, suitable primer and PCR kit (BioNeer kit). PCR product was electrophoretic on the agarose gel, pictured to be assured the success of gene amplification and getting the required portion of DNA of 559 bp (Figure.2).

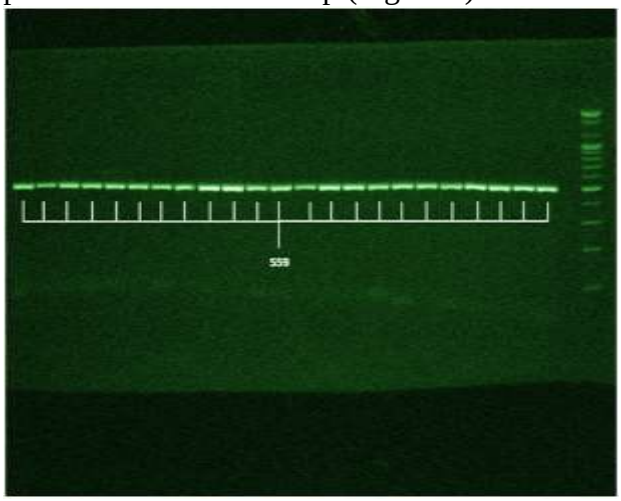

Figure 2. PCR product for the VLDLR gene(fragment 559bp) on agarose gel(1.5%)

SNP identification and genotyping by PCR-RFLP

The genotype of the VLDLR gene polymorphisms were analyzed using PCR-RFLP method. This method was performed by mixing 10 µl of PCR product with 1 U of restriction enzyme (Eco57I), 2 ml buffer y, 0.5 SAM buffer and 0.5 BAS buffer and incubating for 3 hours at 37º C. The product was electrophoresed on 3% agarose gel and stained with ethidium bromide for visualization product. Two PCR fragments for each locus was obtained in this population (Figure 3)

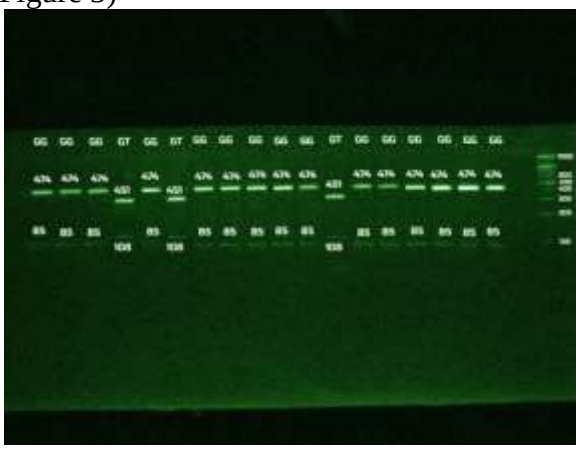

Figure 3. *Eco571*– PCR- RFLR genotyping of chicken VLDLR gene, GG=474 and 85 bp and GT= 108 bp

The genotypic and allelic frequencies of VLDLR in Iraqi local Brown Chickens

Table 1 shows the genotypic and allelic frequencies of single nucleotide polymorphisms (SNP) of the VLDLR gene for 173 local hens. The genotypic frequency was 94.7 and 5.3% for genotypes GG (164 birds) and GT (9 birds) respectively. On the basis of the chi-square test, data analysis did not exhibit an agreement with Hardy-Weinberg equilibrium (P <0.0307) by chi-square test, so that indicate the role of the selection for egg production trait in this studied chicken which leads to increase favorable alleles than others. The current bird's population of this study has been drawn from birds selected for high egg production for three generations (17). Many factors could violate the Hardy-Weinberg equilibrium, especially in small populations, such as intensive selection and genetic drift (9). Previous studies on chickens (25; 27) and quails (24) were also revealed that the low-density lipoproteins receptor (LDLR) and VLDLR genes were not agree with Hardy-Weinberg equilibrium in birds selected for egg production trait for several generations.

Table 2. Genotypic and allelic frequencies of VLDLR gene in Iraqi local Brown chickens

Number of chickens	Genotypic frequency			Allelic frequency		Chi-Square (χ2)	*P*-value
173	GG	GT	TT	G	T		
	94.7	5.3	0	97.4	2.6	4.67	0.0307

Egg production traits

Egg production, egg weight, and egg mass are shown in Table 2. Significant differences (P≤0.0001) in egg numbers for GG genotypes was 98, 81, 59 and 238 eggs per hens during period from 20-40, 40-60, 60-80 and 20 to 80 weeks of age respectively, whereas, hens followed GT genotypes achieved 32, 9, 2 and 43 eggs for the above periods respectively. Furthermore, weekly egg production (%) of both genotypes is presented in Figure 4. Hens followed GG genotypes showed plateau curve shape than those of GT genotypes. Egg weight was higher (P≤0.0001) at 20-40, 40-60 and 20 to 80 weeks of age in GG genotype hens compared with that followed GT genotype (45, 49 and 47 g versus 43, 39 and 43 g respectively). Egg mass of the GG genotype was higher (P≤0.0001) than that of the genotypes (GT). It was 4405, 3976 and 2902 g during periods from 20 to 40, 40 to 60 and 60 to 80 week of age, respectively, while it was 1566, 447 and 127 g for the GT genotypes for the same periods. Total egg mass of GG genotype was 11395 g compared with 2149 g for GT genotypes (Table 2). Egg production and egg weight traits are the most important traits in layers. In this study, hens followed GG genotype exhibited a higher production rate than GT genotype hens that exhibited a greater decline in their production after peak production which refer to impairment in persistence than those of GG genotype. In the GG hens, about 41.1% of total egg production was obtained in the part records periods (20 to 40 week of age) while in GT hens, 74.4% was produced in the same period. That mean hens followed GT genotype has low persistency after peak production. On the other hand, two traits mentioned above were negatively correlated and with continuous selection to increase egg production, the decline in egg weight could occur. Therefore, discovering genetic markers or SNPs affected egg production traits positively in chickens and other domestic fowls are an issue. With respect to the egg production and egg weight, many regions on macro, microchromosomes and Z chromosome has been found to be associated with these two traits (http://www.animalgenome.org, 20). Since most of these regions were population-specific (23), markers would be examined further on another population. The SNPs are extensively used in linkage analysis and variability evaluation in natural populations due to it has reliability in laboratory handling and data interpretation (3). In the present study, the results showed egg production, egg weight, and mass among was significantly affected by SNP variation where hens followed GG-genotypes achieved greater egg performance than those of the GT-genotype, it may be due to a mutation in the VLDLR gene in hens followed GT genotype. In chickens, a point mutation (G/C) was observed at position 2177

bp in the ovulatory restricted gene caused an unpaired of cysteine residue and consequence prevents the accumulation of the yolk proteins precursors in the oocytes and reduced or ceases egg production (7; 14). In the current study, G/T a point mutation was noticed as hens in GT genotype produced little eggs. Previous studies have demonstrated the importance of VLDLR receptor gene in reproduction in chickens (26; 8), zebra finch (10), duck (20) and quail (21). This finding supports the previous results of VLDLR as a compromising gene. The current results approved that the VLDLR gene can be used as a candidate gene for modulating and improving egg production traits in Iraqi localchickens

Table 2. Effect of genotype of the VLDLR gene on egg production, egg weight and egg mass of Iraqi local chicken (mean ± standard error)

Traits	Genotype[1]		*P*-value
	GG (164)	GT (9)	
Egg production (egg/hen), week			
20 to 40	98 ±1.68[a]	32 ±4.22[b]	0.0001
40 to 60	81 ±2.03[a]	9 ± 4.41[b]	0.0001
60 to 80	59 ±2.38[a]	2 ± 4.41[b]	0.0001
20 to 80	238 ±4.73[a]	43 ± 7.69[b]	0.0001
Egg weight (g), week			
20 to 40	45 ± 0.22[a]	43 ± 0.97[b]	0.0001
40 to 60	49 ± 0.37[a]	39 ± 3.71[b]	0.0001
60 to 80	49 ± 0.18	48 ± 0.80	0.5308
20 to 80	47 ± 0.18[a]	43 ± 1.22[b]	0.0001
Egg mass (g/hen), week			
20 to 40	4405 ±72.36 [a]	1566 ±84.89[b]	0.1161
40 to 60	3976 ± 105.23 [a]	447 ± 220.20[b]	0.0001
60 to 80	2902 ± 118.18 [a]	127 ± 50.26[b]	0.0001
20 to 80	11395 ± 230.77 [a]	2149 ± 326.17[b]	0.0001

[a-b] mean within same row have different superscripts are differ significantly (P <0.05).
[1] :number in parentheses represent the number of hens

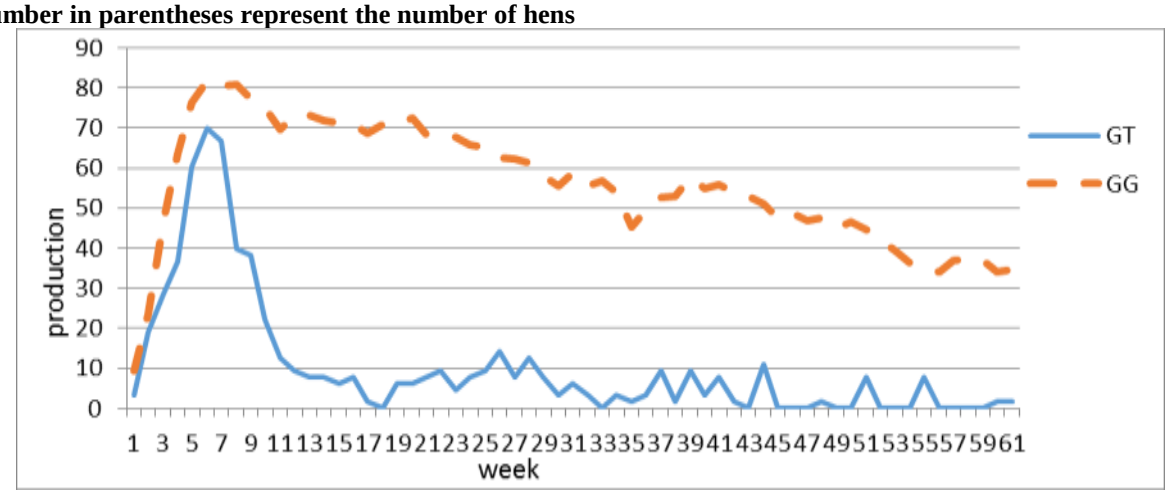

Figure 4. Effect of VLDLR gene on egg production (%) in Iraqi local Brown chickens

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