# VARIATIONS AND EXPRESSION OF VERY LOW-DENSITY LIPOPROTEIN RECEPTOR (VLDLR) mRNA IN THE LIVER AND OVARY OF A LOW EGG PRODUCTION STRAIN OF LOCAL IRAQI HENS

**D. H. Al-Hassani*** **W. M. Razuki**** **H. S. Abdulwahid*****
**Prof.** **Research Director** **Researcher**
***Department of Animal Production, College of Agriculture Engineering Sciences, University of Baghdad.**
****Poultry Research Station, Office of Agricultural Research, Ministry of Agriculture. Baghdad, Iraq**
*****Department of Animal Productions, Ministry of Agriculture. Baghdad, Iraq,**
alhassani.dihya@yahoo.com drwaleedrazuki@yahoo.com heidersattar@gmail.com

## ABSTRACT

**This work was conducted to study the association of variations in the VLDLR gene and mRNA expression in the liver and the ovary of local Iraqi hens with some productive and blood traits. The VLDLR gene variations were studied using blood samples of 173 pedigreed hens, 47 weeks old while the expression was studied using tissue samples of the ovary and liver in 30 pedigreed hens, 60 weeks old. Genotypic and allelic frequencies, production traits, and biochemical serum blood 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 and egg weight for the groups of birds that follow the GG genotypes compared to those that follow the genotypes GT. High levels of VLDLR mRNA were present in the liver and the ovary of high-egg-yield layers but were absent or rare in hens with low-egg production rates. The high expression of VLDLR mRNA caused a reduction in serum triglyceride; LDL and VLDL compared with a low expression where showed increases in these traits while other traits were not affected between different levels of expression. This study suggests that the expression level of VLDLR may be a new molecular marker to screen for high egg production in low-egg production local chickens.**

**Kew words: VLDLR mRNA, liver, ovary, Iraqi local chickens, performance**



# التغاير والتعبير الجيني لمستقبل البروتينات الدهنية واطئة الكثافة جدا للرنا الرسولي VLDLR Mrna في كبد ومبيض سلالات الدجاج المحلي العراقي المنخفض الانتاج

**ضياء حسن الحسني* وليد محمد رزوقي** حيدر ستار عبد الواحد****
استاذ رئيس باحثين باحث علمي
*قسم الانتاج الحيواني–كلية علوم الهندسة الزراعية ** وزارة الزراعة–دائرة البحوث الزراعية
*** وزارة الزراعة–قسم الانتاج الحيواني**

## المستخلص

**اجريت هذه الدراسة بهدف التعرف على دراسة العلاقة بين مستقبل جين VLDLR وتعبير mRNA في الكبد والمبيض لدى الدجاج المحلي العراقي وتاثيراتهما في بعض صفات الإنتاج و الدم. تمت دراسة الاختلافات في VLDLR باستخدام عينات دم لـ 173 دجاجة منسبة بعمر 47 أسبوعًا، بينما تمت دراسة التعبير باستخدام عينات نسيج المبيض والكبد في 30 دجاجة بعمر 60 أسبوعًا. تم فحص التكررات الوراثية والأليلة ، وصفات الإنتاج ، ودم المصل الكيميائي الحيوي. أظهرت النتائج أن التكررات الوراثية والأليلية لمستقبل جين VLDLR في 173 دجاجة لا تتفق مع توازن هاردي واينبرغ (P <0.0307)كانت التكرارات الوراثية 94.7 و 5.3٪ لكل من الطرز الوراثية GG و GT على التوالي بينما كان التكرار الأليلي للجينين G و T 97.4 و 2.6% على التوالي.. أظهرت النتائج زيادة (P <0.0001) في إنتاج البيض ووزن البيض لمجموعات الطيور التي تتبع الطرز الوراثية GG مقارنة بتلك التي تتبع التراكيب الجينية GT. كما اتضح ان المستويات العالية من VLDLR mRNA كانت اكثر تعبيرا في كبد ومبيض الدجاجات مرتفعة الانتاج من البيض، غير انها تكاد تكون غائبة أو نادرة في الدجاج ذي الانتاج المنخفض من البيض. ادى التعبير المرتفع لـ VLDLR mRNA الى خفض مستوى الدهون الثلاثية في الدم، الروتينات الدهنية واطئة الكثافة( LDL) وواطئة الكثافة جدا ( VLDL ) مقارنة بالتعبير المنخفض الذي أظهر زيادات في هذه الصفات، بينما لم تتأثر الصفات الأخرى بين مستويات التعبير المختلفة. تقترح الدراسة الحالية ان مستوى التعبير الجيني لمستقبل جين VLDLR قد يعد مؤشر جزيئي جديدة للكشف عن ارتفاع إنتاج البيض في الدجاج المحلي منخفض إنتاج البيض.**

**الكلمات المفتاحية: البروتينات الدهنية واطئة الكثافة جدا للرنا الرسولين الكبد، المبيض، الدجاج المحلي العراقي، الاداء الانتاجي**
**Received:28/5/2021, Accepted:15/8/2021**

## INTRODUCTION

In mature laying chickens, egg production process stimulates by estrogen mediates marked increases in hepatic lipid production and changes in the diameter of assembled very-low-density lipoprotein (VLDL) shifted from generic VLDL (~70 nm in diameter), which transports lipids to peripheral tissues, to yolk-targeted VLDL (VLDLy; ~30 nm) (19). The liver is the major site of lipoproteins and yolk precursors synthesized and secretion. The growing oocytes are taken these metabolites through endocytosis processes involving specific cell-surface receptors (20). Yolk lipids transport to the oocytes by VLDL yolk-targeted (VLDLy), which have a specific receptor (VLDLR) in the ova surface. VLDLR is a transmembrane lipoprotein receptor of the low-density-lipoprotein (LDL) receptor family, it's abundant in skeletal muscle, adipose tissue, heart, and the brain, but it is absent from the liver (16). VLDLR is also named vitellogenesis receptor (OVR) or vitellogenin receptor (VTGR), mediated the absorption of yolk proteins from plasma very-low-density lipoproteins and vitellogenin (23), It also acts as a part of triglycerides and cholesterol metabolism (5), as well as in many cellular processes of cell proliferation, migration, and differentiation (11). VLDLR in chicken (4; 21) and other oviparous species (10; 23) 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. In contrast, Wang *at el*., (24) showed that the expression level of VLDLR mRNA in the high egg production breeds were not necessarily associated with enhanced VLDLR expression. The ovary also plays an important role in depositing lipids in the developing yolk through receptor-mediated action Formation of poultry eggs requires the orchestration of genes/proteins expression in a spatial and- temporal manner in the oviduct, along with the optimal synthesis of reproductive hormones. Many of studies have shown the importance of very low- density lipoprotein (VLDLR) gene as a candidate gene to modulate egg production or growth in chickens (1; 24), zebra finch (10), ducks (14; 23) and quail (27). Local chickens in many developing countries are still producing fewer eggs than standard commercial chickens; for example, the commercial layers produce more than 300 eggs per hen in a year compared with as much as 200 eggs or less for local egg-type chickens which depends on the breed and country. The reduction in egg output occurred sharply soon after peak production, more reduction in some birds and cessation in others, was noticed in local Iraqi chickens. Nevertheless, the Iraqi local chicken is one of the most important genotypes that must be preserved and conserved, which represents gene bank of adapted and resistant traits to Iraqi harsh environment (2; 17). In respect to the local breeds, a relationship between deferent breeds of chicken and expression of genes related with egg performance were noticed (8; 22). An experiment was conducted to assess the association between VLDLR gene expression in ovary and liver of aged-laying hens with egg performance and biochemical properties in local Iraqi Brown chickens selected for high egg production for three successive generations**.**

## MATERIALS AND METHODS

### Birds and Data collection

One hundred and seventy-three Iraqi Brown native chickens were used in the genotyping study, but in the gene expression, the data were taken from thirty hens according to their egg production. Hens were kept in an individual cage and their egg production was recorded daily from onset egg to 60 weeks of age. Egg number and egg weight for each hen was recorded daily and summarized in for the period, 20 to 40, 40 to 60, and 20 to 60 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 free. Blood samples were drawn from wing vein of 47 weeks old hen by collecting 3 ml of each inside test tubes containing EDTA anticoagulant for DNA isolation and without EDTA for serum biochemical study of glucose, triglyceride, cholesterol, HDL, LDL, VLDL and total

protein using specific kits according to the manufacturer protocol (Spinreact-Spin Company, GIRONA; Spain). The samples were placed inside the refrigerated box and immediately stored at -20 º C until laboratory analysis. Chicken genomic DNA was extracted from the blood by a phenol/chloroform method. The primers and restriction enzymes used for polymerase chain reaction (PCR) are presented in Table 1. 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. Restrict ovulatory (RO) mutation was identified using PCR-RFLP. A total of 173 hens descending from third generations of local Iraqi chickens select for high egg production were used. A 559 bp fragment containing the RO locus was obtained by PCR using the forward primer 5'-TCTATGGTGCCAACAAAT-3' and the reverse primer 5'-CATCTCAGACCGTCCTCC-3'. After digestion of the PCR products with *Eco*57I (Bioneer, South Korea) at 37 °C for 2 h, the products were separated on a 2% agarose gel.

**Tissue samples, cDNA isolation, cDNA synthesis and DNA synthesis :** The levels of VLDLR mRNA expression in ovary and liver of the local Iraqi chickens at 60 week-old hens were detected. Thirty hens from suggested population (173 hens) were selected based on their egg number records; group 1, represent birds with the egg number exceed the flock average, group 2 represent birds with the egg number at the flock average, and group 3, represent birds with the egg number less than 50 eggs or those no producing eggs. The birds were killed gently by knife (Halal method). The tissues of liver and ovary with previtellogenic follicles (diameter < 5 mm) were immediately removed and immersed in RNA later solution (Geneaid, South Korea) at 4 °C overnight and then stored at −80 °C until used as described by Wang et al., (24). Total RNA was extracted from tissues using Accuzol Trizol according to the manufacturer's protocol (Geneaid, South Korea). The quantity and quality of extracted RNA were confirmed with NANODROP 2000c (ACT gene, USA). Ratios of A260/280 ranged from 2.0 to 2.2, suggesting a high level of purity among all RNA samples. In order to synthesize the first-strand complementary DNA fragment, 18 µL of RNA was added to the tube containing Accupower® rocket Script $^{TM}$ RT Premix, 2 µL of oligonucleotide was added to the mixture, the mixture was mixed by using the Exispin for 6 cycles and then the mixture was incubated with thermal cycler DNA incubator. The standard curves were created with a five-fold dilution series of cDNA as a template for each treatment using a linear regression model. The cDNA of all samples was stored at -20°C. The expression of VLDLR in ovary and liver was detected using quantitative real-time PCR (qPCR). The reactions were run using Accupower® GeenStar $^{TM}$ qPCR Premix kit, (Bioneer, Soul, South Korea), in total volume of 20 µL containing 4 µL of cDNA , 2 µL of primer pairs (The forward and reverse primers were 5'-TGTGGTCCTCAGTCAACC-3' and 5'-TCTGCTGCACTACAAGTCA-3' for VLDLR and 5'-ATACACAGAGGACCAGGTTG-3' and 5'-AAACTCATTGTCATACCAGG-3' for GAPDH (23) and 14 µL DEPC- distilled water . The amplifying conditions of PCR for normal DNA were 94°C for 5 min, followed by 40 cycles of 95°C for 30 s. The annealing temperature used to amplify the bisulfite-treated DNA was 60°C. The PCR products were visualized by electrophoresis on 1.5% agarose gel electrophoresis**.** The reactions were run in a Qiagen Rotor-Geeneq real-time PCR detection platform (The Lab World Group, Germany) using the cycling conditions described in the UltraSYBR mixture protocol sheet. Two technical replicates were run for every sample.

**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 and egg weight in Iraqi native 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). The resulting Ct data were analyzed using the $2^{-\Delta\Delta CT}$ methods (12). GAPDH was used as a housekeeping gene to normalize the amount of cDNA input. Figures were drawn through using Excel data sheet.

**RESULTS AND DISCUSSION**

A 559 base pair (bp) of VLDLR was primarily obtained by PCR -RFLP technique (Figure 1a). Two PCR fragments for each locus was obtained in this population, the wild type was labelled with two bands, 474 and 85 bp and another genotype was also labelled with two bans, 451 and 108 bp (Figure 1b). Since restrict ovulate (RO) mutant gene should show four bands (85 bp, 474 bp, 108 bp and 451 bp), the mutant gene was not considered and comparison was made between a group of hens that exhibited different genotyping polymorphisms. Table 1 shows the genotypic and allelic frequencies of single nucleotide polymorphisms (SNP) of the VLDLR gene for 173 native hens. The genotypic frequency was 94.7 and 5.3% for genotypes GG (164 birds) and GT (9 birds) respectively. Based on 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 level of VLDLR mRNA expression was compared in three groups of hens (high, moderate and high egg production). RT-PCR analysis of VLDLR mRNA was predicted to generate a fragment of 1474 bp, we found that the chicken VLDLR gene was highly expressed in the liver and in the ovary of hens followed high egg production rate than those of low-egg production rate (Fig. 2 a, b). We also observed that VLDLR was over-expressed in the liver of the highly egg production hens compared with the ovary. In contrary, the VLDLR expression in the liver of the low egg production hens was much lower compared the ovary. On the other hand, the values of the relative expression of VLDLR mRNA to reference gene (GAPDH) tend to be same ($P>0.05$) in three groups of hens with slightly increases in the group of hens followed low-egg production rate (Fig 3). Egg performance is shown in Table 2. Age at first egg and first egg weight were not affected by SNP variation. Highly significant differences ($P\leq0.0001$) in egg numbers and mean egg weight were showed between genotypes. Hen with GG genotype had higher egg numbers and egg weight at different period studied. Serum blood biochemical parameters are presented in Table 3. Serum cholesterol ($P<0.0831$) and LDL ($P<0.0190$) was increased in hens flowed GT genotype, whereas, other parameters were not different between genotype differences. Table 4 shows the association of expression of the VLDLR mRNA with egg production traits. A highly significant association ($P < 0.0001$) was found between the expression and egg number and egg weight. The high levels of expression of VLDLR mRNA in the liver and ovary lead to an increase in total egg number and average egg weight from 20 to 60 weeks of age. Besides, age at first egg occurred about nine days earlier in hens showed high VLDLR mRNA expression than those in low or moderate expression. The high expression of VLDLR mRNA caused a reduction in serum triglyceride; LDL and VLDL compared with a low expression where showed increases in these traits while other traits were not affected between different levels of expression (Table 5). The current bird's population of this study has been drawn from birds selected for high egg production for three generations. Many factors could violate the Hardy-Weinberg equilibrium, especially in small populations, such as intensive selection and genetic drift (9; 14; 26). Previous studies on chickens (28; 30) and quails (27) were also revealed that the low-density lipoproteins receptor (LDLR) and VLDLR genes were not agreed with Hardy-Weinberg equilibrium in birds selected for egg production trait for several generations. Concerning the egg production and egg weight, many regions on macro, microchromosomes and Z chromosome is

associated with these two traits (http://www.animalgenome.org, Wolc *et al*., (25). Since most of these regions were population-specific, 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 the laboratory handling and data interpretation (1). 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 (6; 15). In the current study, most individuals genotyped showed two bands (85 and 474 bp) but, also we found ten hens of the total sample (173 hens) have two different bands (451 and 108 bp) where they produced less than 50 eggs per hen. Egg production and egg weight traits are the most important traits in layers. In the present study, the results showed egg production and egg weight was significantly affected by SNP variation where hens followed GG-genotypes achieved greater egg performance than those of the GT-genotype; it might be due to a mutation in the VLDLR gene in hens followed GT genotype. Also, we noticed that hens followed GT genotype exhibited a greater decline in their egg production after peak production which refers to impairment in persistence than those of GG genotype. In the GT hens, about 41.1% of total egg production was obtained in the part records periods (20 to 40 week of age) while in GG hens, 74.4% was produced in the same period. That mean hens followed GT genotype has low persistency after peak production. On the other hands, two traits mentioned above were correlated negatively and with continuous selection to increase egg production, the decline in egg weight could occur. The current result consistency that both egg production and egg weight traits were positively improved in a group of hens followed GG-genotype. Previous studies have demonstrated the importance of VLDLR receptor gene in reproduction in chickens (7; 29), the zebra finch (10), duck (23) and quail (27). This finding supports the previous results of VLDLR as a compromising gene. The level of VLDLR mRNA expression in the ovary and liver was higher in the group of hens that produced 200 eggs or higher for 40 weeks of production as a fold change of control group. Whereas, hens that produced less than 50 eggs for the same period expressed lower than the control group (Table 4). This result revealed that the level of VLDLR mRNA expression in the ovary was a determining factor in the reproductive phenotype, as shown by Han *et al*. (10).While Wang *et al*. (24) showed that the expression level of VLDLR mRNA in the high egg production breeds were not necessarily associated with enhanced VLDLR expression. In the current study, high and low levels of VLDLR mRNA expression were noticed in the ovary and liver. Interestingly, we observed over- and low-expression in the liver of the high and the low egg production hens respectively, compared with the ovary (Figure 2 a, b). The results suggest that the liver of hens is the major site for the production of VLDL yolk targeted in spite of the crucial role of the ovary to participate to deposit and accumulate yolk precursors in the growing oocytes through receptor-mediated action. Another clue to the function and expression of the VLDLR came from analysis of the triglyceride (TG), LDL and VLDL which increases in serum of hens produced lower eggs and expressed at a low level of VLDLR (Table 5). Hyperlipidemia and higher abdominal fat bad (data not shown) in hens with a low level of VLDLR mRNA may refer to the female sterility due to mutant oocyte VLDLR gene. In conclusion**,** our data indicate that VLDLR variants tend to be fixed in the selected breeds. The higher egg production rate associated with VLDLR may be a result of greater hepatic lipoprotein synthesis and involvement through endocytosis process in the selected hens for high egg production. We observed over- and low-expression in the liver of the high and the low egg production hens respectively, compared with the ovary. Our results indicate that the VLDLR can be used as a genetic marker for improving the egg production in the local Iraqi brown chickens.

**Acknowledgements:** The authors would like to thank Office of Agricultural Research for providing all field materials and College of

Agriculture Engineering Science for providing valuable help for this study.

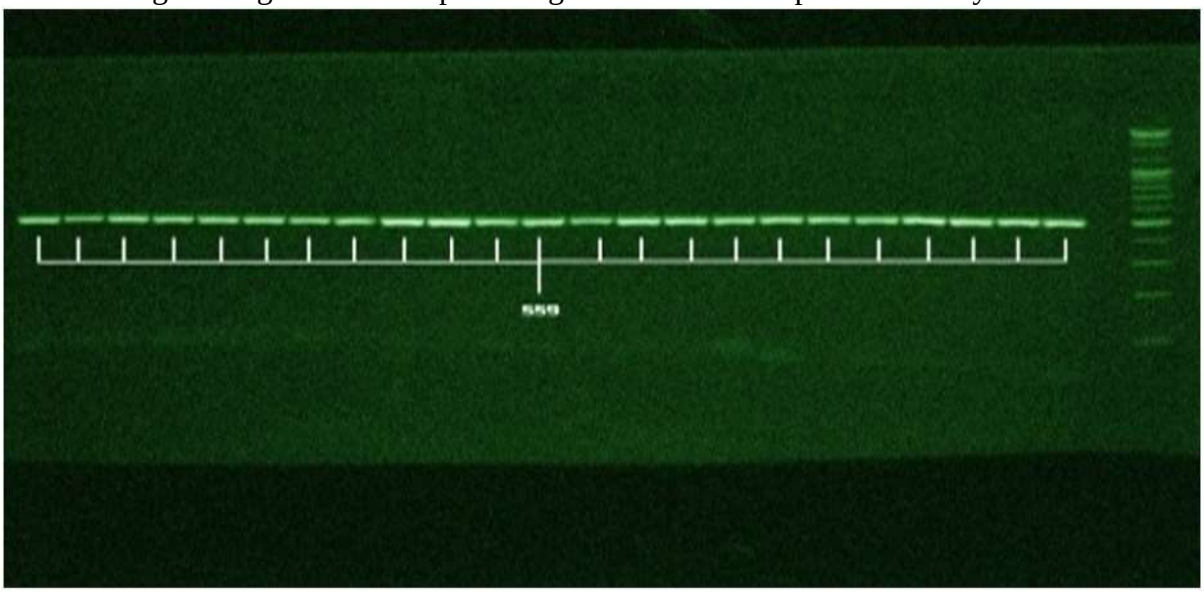


**Figure 1a. Gene Extraction of the VLDLR gene using the PCR technique, samples of DNA product, suitable primer and PCR kit (BioNeer kit). The PCR product was electrophoretic on the agarose gel, pictured to be assured the success of gene extraction and getting the required portion of DNA of 559 bp.**


**Figure 1b. PCR- RFLP genotyping of local Iraqi Brown chicken VLDLR gene using *Eco*57I restricted enzyme. GG is a wild-type genotype has two bands (474 and 85 bp) and GT is nominated as a mutant genotype has two other bands (451 and 108).**

**Table 1. Genotypic and allelic frequencies of VLDLR gene in Iraqi Brown native chickens**

| Number of chickens | Genotypic frequency | | Allelic frequency | | Chi-square ($\chi 2$) | *P*-value |
|---|---|---|---|---|---|---|
| | GT | GG | T | G | | |
| 173 | 94.7 | 5.3 | 97.4 | 2.6 | 4.67 | 0.0307 |

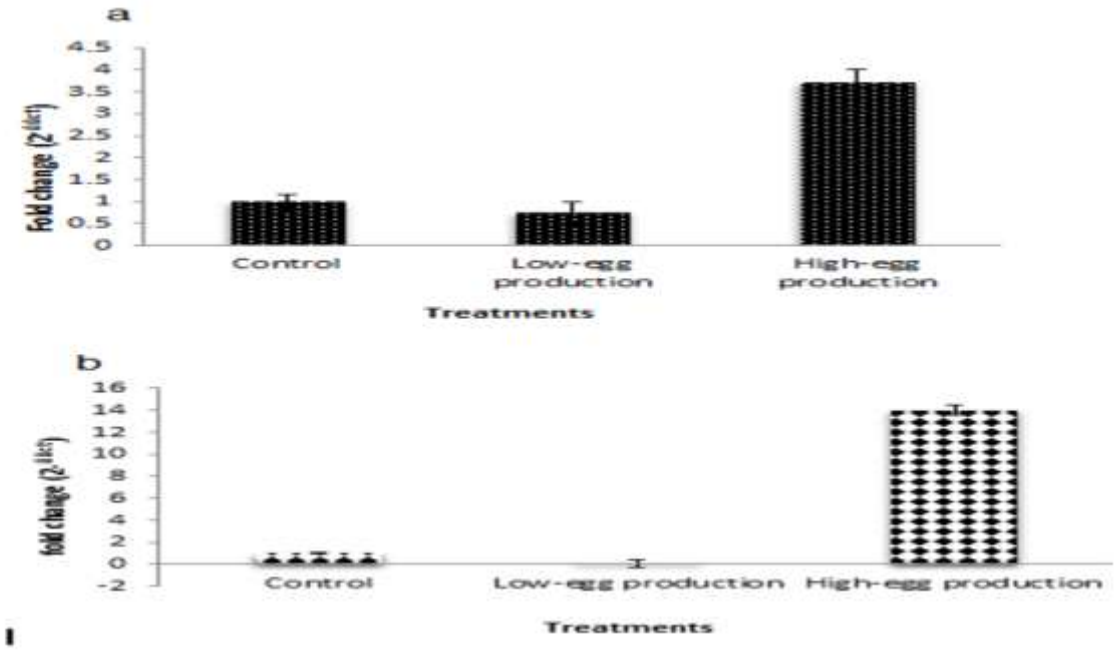


**Figure 2. The VLDLR mRNA expression in the ovary (a) and liver (b) of aged laying hens Quantitative Real-time PCR was carried out; GAPDH is a reference internal gene. Vertical lines represent the SEM**

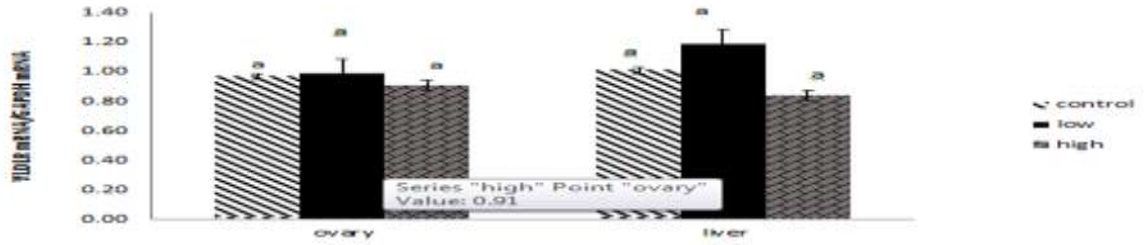


**Figure 3. The VLDLR mRNA to GAPDH expression ratio in the ovary (a) and liver (b) of aged laying hens. Quantitative Real-time PCR was carried out; GAPDH is a reference internal gene. Vertical lines represent the SEM, and the same letters indicate non-significant differences among the mean (p>0.05).**

**Table 2. Association of the SNPs in the VLDLR gene with egg production traits of local Iraqi Brown Chickens**

| Traits | Genotype[1] | | *P*-value |
|---|---|---|---|
| | GG (164) | GT (9) | |
| Age at first egg (day) | 151.6± 3.11 | 154.9± 1.44 | 0.5987 |
| First egg weight (g) | 36.1± 3.01 | 37.5± 0.40 | 0.4712 |
| 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 |
| 20 to 60 | 179 ± 2.89[a] | 41 ± 7.00[b] | 0.0001 |
| Mean 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 |
| 20 to 60 | 47 ± 0.23[a] | 41 ± 1.76[b] | 0.0001 |

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

**Table 3. Association of the SNPs in the VLDLR gene with serum blood biochemical parameters of local Iraqi Brown Chickens**

| Traits | Genotype | | P-value |
|---|---|---|---|
| | GG (163) | GT (9) | |
| Glucose (mg/dl) | 310.6 ± 4.7 | 331.7± 17.8 | 0.3038 |
| Cholesterol (mg/dl) | 305.0 ± 5.1 | 343.6 ± 19.5 | 0.0831 |
| Triglyceride (mg/dl) | 966.5 ± 10.7 | 994.0 ± 23.0 | 0.5508 |
| HDL (mg/dl) | 41.4 ± 1.4 | 44.3 ± 4.9 | 0.6365 |
| LDL (mg/dl) | 79.2 ± 3.8[b] | 119.2 ± 17.8[a] | 0.0190 |
| VLDL (mg/dl) | 193.4 ± 2.1 | 198.8 ± 4.6 | 0.5531 |
| Total protein (g/L) | 12.4 ± 0.2 | 12.4 ± 0.9 | 0.9795 |
| Albumin (g/L) | 2.1± 0.03 | 2.2 ± 0.1 | 0.8337 |

[a-b] mean within the same row have different superscripts differ significantly (P <0.05).

[1]: number in parentheses represents the number of hens

**Table 4. Association of the Levels of expression of VLDLR mRNA gene in the liver and ovary of hens with egg production traits**

| | RNA expression groups | | | |
|---|---|---|---|---|
| traits | Low | control | high | P-value |
| Age at first egg (day) | 151±3.64[a] | 150±2.60[ab] | 142±1.73[b] | 0.0620 |
| 1st. egg weight (g) | 35.2±72 | 36.2± .10 | 33.7± .95 | 0.1719 |
| | Egg number per bird, week | | | |
| 20-40 | 29.7±4.63[c] | 103.6±1.89[b] | 126.1±1.78 | <0.0001 |
| 40-60 | 15.2±9.92[b] | 86.8±3.24[a] | 95.1±6.17[a] | <0.0001 |
| 20-60 | 44.9±9.60[c] | 190.4±3.17[b] | 221.4±7.19[a] | <0.0001 |
| | Egg weight, g, week | | | |
| 20-40 | 43.3±0.86 | 45.3±1.25 | 44.3±0.82 | 0.4257 |
| 40-60 | 39.1±3.84[b] | 48.3±0.69[a] | 48.1±1.09[a] | 0.0057 |
| 20-60 | 41.0±1.90[b] | 46.8±0.68[a] | 46.2±0.62[a] | 0.0019 |

[a-b] mean within the same row have different superscripts differ significantly (P <0.05).

**Table 5. Association of the Levels of expression of VLDLR mRNA gene in the liver and ovary of hens with serum blood biochemical parameters**

| traits | RNA expression groups | | | *P*-value |
|---|---|---|---|---|
| | Low | Control | High | |
| Glucose (mg/dl) | 335± 9.48 | 318± 18.3 | 318± 17.9 | 0.6478 |
| Cholesterol (mg/dl) | 330±20.6 | 282± 19.9 | 316± 19.8 | 0.2312 |
| Triglyceride (mg/dl) | 1015± 28.3[a] | 937± 25.3[a] | 893± 27.8[b] | 0.0126 |
| HDL (mg/dl) | 39.3± 4.6 | 47.1± 8.17 | 40.1± 4.8 | 0.6129 |
| LDL (mg/dl) | 103± 18.0[a] | 53.4± 9.3[b] | 86.0± 13.8[ab] | 0.0578 |
| VLDL (mg/dl) | 203± 5.7[a] | 187± 5.07[ab] | 178± 5.6[b] | 0.0126 |
| Total protein (g/L) | 11.7± 0.67 | 12.4± 0.52 | 11.1± 0.41 | 0.2748 |
| Albumin (g/L) | 2.32± 0.40 | 2.25± 0.07 | 2.24± 0.08 | 0.8064 |

[a-b] mean within the same row have different superscripts differ significantly (P<0.05).

## REFERENCES

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. Iraqi J. Agric. Sci. 50(2):727 – 733

2. Al-Hassani D.H., 1991. Effect of heat stress on some physiological characteristics of Iraqi indigenous chickens. Iraqi J. Agri. Sci. 22, 2:77-82.

3. Ane X. P., J. X. Hou, G. Li, Y. X. Song, J. G. Wang, Q. J. Chen, Y. H. Cui, Y. F. Wang,and B. Y. Cao. 2011. Polymorphism identification in the goat *KITLG* gene and association analysis with litter size, Anim. Genet., 43: 104-107.

4. Barber D. L., E. J. Sanders, R. Aebersold, W. J. Schneider. 1991.The receptor for yolk lipoprotein deposition in the chicken oocyte. J. Biol.l Chem., 266:18761-18770

5. Brown M.S.,and J. L. Goldstein.1986. A receptor-mediated pathway for cholesterol homeostasis. Science, 232:34-47.
6. Bujo H., T. Yamamoto, K. Hayashi, M. Hermann, J. Nimpf, and W. J. Schneider.1995. Mutant oocyte low-density lipoprotein receptor gene family member causes atherosclerosis and female sterility. Proceed. Natl. Acad. Sci. USA, 92(21): 9905-9909
7. Cao D. G., Y. Zhou, Q. Lei, H. X. Han, F. W. Li, G. M. Li, Y. Lu, B. Wu, and Z.L. Wang. 2012. Association of very-low-density lipoprotein receptor VLDLR gene polymorphisms with reproductive traits in a Chinese indigenous chicken breed. J. Anim. Vet. Adv.,11:3662-3667
8. Ding S..T, Y. H. Ko, B. R. Ou, P. H. Wang, C. L. Chen, M. C. Huang, Y. P. Lee, E. C. Lin, C. F. Chen, H. W. Lin, and W. T. K. Cheng. 2008.The expression of genes related to egg production in the line of Taiwan country chickens. Asian Austr. J. Anim. Sci., 21:19-24
9. Falconer D. S., 1989. *Introduction to Quantitative Genetics*, Ed. 3. Longmans Green/John Wiley & Sons, Harlow, Essex, UK/New York
10. Han, D. N., H. Haunnel, and T. D. Williams. 2009. Variation in yolk precursor receptor mRNA expression a key determinant of reproductive phenotype in the zebra finch *Taeniopygia guttata*. J. Exp. Biol., 212: 1277-1283
11. Hussain, M. M. 2001. Structural, biochemical and signaling properties of the low-density lipoprotein receptor gene family. Front Biosci., 6 :D417-D428.
12. Livak K. J., and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T) Method. Methods. 25(4):402-8. doi: 10.1006/meth.2001.1262. PMID: 11846609
13. Meng X. T., N. N. Hou, X. J. Wang, H. C. Jiao, J. P. Zhao, Z. G. Song, and H. Lin. 2013. Increased hepatic yolk precursor synthesis ، secretion and facilitated uptake by follicles are involved in the rejuvenation of reproductive performance of moulted hens (*Gallus gallus nativeus*). Genet. Comp. Endoc., 194:198-207
14. Ming-liang C., W. Cong, D. Xuan, L .Lu, and Z. Tang-Jie. 2017. A novel polymorphism of VLDLR signal peptide coding region and its association with growth and abdominal fat traits of Gaoyou. domestic ducks. Braz. J Poult. Sci, 19(4):577-582
15. Nimpf, J. and W. J. Schneider, 1998. The VLDL receptor: an LDL receptor relative with eight ligand binding repeats, LR8. Atherosclerosis, 141(2):191-202
16. Nimpf J., and W. J. Schneider. 2000. From cholesterol transport to signal transduction: low density lipoprotein receptor, very low density lipoprotein receptor, and apolipoprotein E receptor-2. Biochimica et Biophysica Acta, 1529(1/3):287–298
17. Razuki W. M., and S. A. Al-Shaheen. 2011. Use of full diallel cross to estimate crossbreeding effects in laying Chickens. Int. J. Poult. Sci., 10(2):87-92
18- SAS Institute. 2002. SAS User Guide for Personal Computer, Release 9.12. SAS Institute, Inc. Cary, NC;
19. Salvante K. G., G. L. Rosemary, L. Walzem, and T. D. Williams. 2007. Characterization of very-low-density lipoprotein particle diameter dynamics in relation to egg production in a passerine bird. J. Exp. Biol., 210: 1064-1074
20. Schneider W. J. 2016. Lipid transport to avian oocytes and to the developing embryo. J. Biomed. Res., 30(3):174-180
21. Shen X, E .Steyrer, H. Retzek, E. J. Sanders,and W. J. Schneider.1993. Chicken oocyte growth: receptor-mediated yolk deposition. Cell Tissue Res., 272:459-471
22. Sun D. X., W. Dong, Y. Xing, and Y. Yuan. 2005. Cloning and characterization of liver cDNAs that are differentially expressed between chicken and their parents. Asian-Australas J. Anim. Sci., 18:1884-1890
23. Wang C, S. J. Li, W. H. Yu, Q. W. Xin, C. Li, Y. P. Feng, X peng and Y. Gong. 2011. Cloning and expression profiling of the VLDLR gene Associated with egg performance in Duck (*Anas platyrhynchos*). Genet. Sel. Evol., 43:29. http://www.gsejournal.org/content/43/1/29
24. Wang Z, G .Meng, N. Li, M .Yu, X . Liang, Y. Min, F. Liu, and Y. Gao. 2016. The associationof very-low-density lipoprotein receptor (VLDLR) haplotypes with egg production indicate VLDLR is a candidate gene for modulating egg production. Genet. Mol. Biol., 39(3):380-391

25. Wolc, A., J. Arango, T. Jankowski, I. Dunn, P. Settar, J. E. Fulton, N. P. O'sullivan, R. Preisinger, R. L. Fernando, D. J. Garrick, and J, C. M. Dekkers, 2014. Genome-wide association study for egg production and quality in layer chickens. J. Anim. Breed. Genet.131 (3):173-182

26. Wu Y, A. L. Pan, J. S. Pi, Y. J. Pu, J. P. Du, Z. H. Liang, and J. Shen. 2012. One novel SNP of growth hormone gene and its associations with growth and carcass traits in ducks. Mol. Biol. Reports, 39(8):8027-8033. Doi: 10.1007/s11033-012-1649-1. Epub 2012 May 1. PMID: 22547272

27. Wu, Y. J. S. Pi, A. L. Pan, J. P. Du, J. Shen, Y. J. Pu and Z. H. Liang. 2015. Two novel linkage SNPs of VLDLR gene intron 11 are associated with laying traits in two quail populations. Arch. Anim. Breed., 56 (1-6):2015, dio:10.5194/aab-58-1-2015

28.Yao, J.F., Z. X. Chen, G. Y. Xu, X. L. Wang, Z. H. Ning, J X. Zheng, L. J. Qu. and N. Yang, 2010. Low-density lipoprotein receptor-related protein 8 gene association with egg traits in dwarf chickens. Poult. Sci., 89(5): 883-886

29. Zhan, H., Y. Feng, S. Shen, P. Gong, X. Peng, S. Li and Y. Gong. 2009. Polymorphisms of chicken oocyte vitellogenesis receptor gene (ovr) and association with egg traits of chickens. J. Agric. Biotech. 17(6): 967-971

30. Zhang, Y., Z. C. Hou, Z. X. Chen, J. X. Zheng , S. R. Chen, L. J. Qu, J. Y. Li, G. Y. Xu. and N. Yang, 2011. Low-density lipoprotein receptor-related protein 2 gene is associated with egg-quality traits in dwarf layers. Poult. Sci., 90(12):.2718-2722.