LEPTIN RECEPTOR GENE POLYMORPHISMS AND Its EFFECT ON SOME PRODUCTIVE AND PHYSIOLOGICAL TRAITS IN LOCAL IRAQI CHICKEN

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ABSTRACT

This study was conducted in poultry farm in college of agricultural engineering Sciences - University of Baghdad - animal production department. In this study 100 laying hens of local Iraqi chickens were used with age 67day old, were placed in individual cages, each cage was numbered from 1 to 100, for the period from 26/10/2021 till 24/7/2022 to detect the different genotypes of LEPR gene, determining the frequency and percentage of the relevant genotypes local Iraqi chickens, , Productive traits were measured from the sexual maturity in to 100 days for each chicken, the blood samples were collected from 100 laying hens at the 38 weeks of age from the brachial vein, the PCR reaction will done using specific primers, Then sanger sequencing technique were done and three genotypes of leptin receptor gene were obtained TT genotype (wild) , TC (heterozygous) and CC genotype(mutant), the C allele frequency were 0.74 had high significant difference ($p<0.01$) as compared with T allele were 0.26 ,there were significant effects ($p<0.05$) between the various genotypes on the feed intake showed CC genotype had the high mean, followed by TC then TT, in 1,2 and 6 periods. The CC genotype had high significant effect ($p<0.01$) and significant effect ($p<0.05$) on albumin and HDL concentration respectively followed by TC genotype then TT genotype, The CC genotype affect significantly on maturity age.

Key words: PCR, T16297C SNP, Sanger method, HDL, maturity.

التغيرات الوراثية لجين مستقبل اللبتين و تاثيرها في بعض الصفات الانتاجية والفسلجية للدجاج المحلي العراقي.

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المستخلص

اجريت هذه الدراسة في حقل الدواجن التابع لكلية علوم الهندسة الزراعية– قسم الانتاج الحيواني– جامعة بغداد على 100 دجاجة بياضة من الدجاج المحلي العراقي، بعمر 67 يوماً. تم وضعها في أقفاص فردية وتم ترقيم كل قفص من 1 إلى 100 ، للفترة من 2021/10/26 حتى 2022/7/24 لغرض الكشف عن التراكيب الوراثية المختلفة لجين مستقبل اللبتين وتحديد التكرار الاليلي والنسبة المئوية للتراكيب الوراثية المختلفة لجين اللبتين في الدجاج المحلي العراقي. تم قياس الصفات الإنتاجية من عمر النضج الجنسي لغاية 100 يوم لكل دجاجة، حيث تم جمع عينات الدم من 100 دجاجة بياضة بعمر 38 أسبوعًا من الوريد الجناحي ,تمت عملية استخلاص الحمض النووي ثم تم اجراء تفاعل الكوثرة باستخدام بادئات متخصصة. بعد ذلك تم عمل تقنية تسلسل سانجر لتحليل تتابعات القواعد النيتروجينية، وتم الحصول على ثلاثة طرز وراثية لجين مستقبل اللبتين النمط الجيني TT (البري)، TC (المتغاير) وCC (الطافر)، وجد فرق عالي المعنوية للتكرار الأليل C الطافر 0.74 ($p<0.01$) بالمقارنة مع الأليل T البري 0.26. كان هناك تأثير معنوي ($p<0.05$) بين الطرز الوراثية المختلفة لجين مستقبل اللبتين على تناول العلف وجد أن اذ بلغ للطراز الوراثي الطافر CC كان له متوسط عالي، يليه TC ثم TT، في الفترات 1،2 و6 ، كان للطراز الوراثي CC لجين اللبتين تأثير عالي المعنوية($p<0.01$) وتاثير معنوي ($p<0.05$) على تركيز الألبومين وHDL على التوالي متبوعًا بالطراز الجيني TC ثم TT ، اظهرت للتركيب الوراثي CC للجين مستقبل اللبتين تأثير معنوي على عمر النضج يليه TC ثم TT .

الكلمات المفتاحية: تفاعل الكوثرة، تعدد اشكال النوكليوتيدات T16297C، تقنية تسلسل سنكر، HDL، النضج الجنسي.

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INTRODUCTION

Poultry production is regarded as one of the most important economies in the world, and the production of table and hatching eggs is one of the most important factors in ensuring internal and global food security. This production requires flocks of highly productive chickens in order to meet the needs of the consumer, whose number increases every year. In Africa and Asia, domestic chickens account for about 80% of chicken flocks (7). Unlike other types of chicken, it is still distinguished by the production of eggs and meat (4). The leptin receptor gene is one of the effective genes that influence the chicken productive and physiological performance, because it's the main role in regulating fat storage and controlling the number of calories storage that eating by chicken. Polymorphisms of leptin receptor gene were carried out by new molecular technology DNA Sequencing and restriction fragment length polymorphism (RFLP), The chicken leptin receptor gene (LEPR), which mediates the physiological effects of leptin, was cloned and sequenced (13,20). the chicken LEPR is located on chromosome 8, Immunization against the extracellular domain of LEPR reduced the LEP signaling pathway in chickens, resulting in decreased feed intake, serum lipid profile values, and egg-laying rates (18). The liver is essential for digestion and metabolism because it regulates the amount of lipids, carbohydrates, and proteins produced, stored, and released, (11). Seroussi (22) were the first to confirm the existence of avian leptin, the leptin gene sequence is exceptionally rich in guanine-cytosine (GC) content (about 70%), with low sequence conservation for both the nucleic and amino acid sequences (30%) and a low expression level (12). The candidate genes are one of the most important factor in selective programs and genetic improvement for chickens and ruminants (1,2,10) Indeed, leptin expression in adipose tissue ranges from undetectable in wild chickens and red jungle fowl to low levels that are unaffected by feed deprivation (5). Leptin is mostly expressed in adipose tissue and the liver in chickens, where the expression of its receptor gene has also been seen (6). Similar to how chicken LEPR was crucial to this signaling pathway that affects the distribution and deposition of fat (11,17), The purpose of this study is to detect the SNP T>16297C in the fifth intron of leptin receptor gene and to discuss their association with some physiological and productive traits in local Iraqi chicken.

MATERIALS AND METHODS

Hens and character measurements: This study was conducted on one hundred chickens, 67 days old, at Department of Animal Production / College of Agricultural engineering science / University of Baghdad, the eggs produced by each chicken were collected, numbered, and weighted individually, and veterinary measures were all carried out in accordance with the program in the location of the breeding of laying chickens and herds of Local Iraqi chickens. This was done in order to record the production of egg per chicken to 100 days (from the age of sexual maturity to the age of 100 Day of production). At first day in the farm water supplied with vitamin C 0.5gm/litter was provided. Throughout the experiment, meals were delivered to the birds, and 100 grams of feed per day was given to and Lohman lighting system were used at all the period of breeding. Each chicken, 31 weeks 5 ml of blood were collected from brachial vein from each chicken, placed in tubes containing an anticoagulant (EDTA) and kept in freezer (-20C^{o}), DNA extraction was done by the Geneaid-Kit Company in Taiwan. At some modification on extraction protocol were done by reducing blood value to 20 ml (20). The genomic DNA Electrophoreses was performed by 1% Agarose gel and 0.2 µl ethidium bromide, then visualized by UV Light, a digital camera was used to get photo for the gel. The PCR technique condition were carried out to target region of LEPR gene by specific primers:

F: 5'- ATGCTGCTTGATTCTTCCTCCT -3'
R:5'-CCCTAGGCAAATGGTAATGAAC -3'

Using the diagnostic kit (GoTaq® Green Master Mix) produced by American Promega with molecular weight 500 bp, the PCR condition was: initial at 94°C for 4 min, then 35 cycles of initial denaturation at 94°C for 30s, annealing at 58.7°C for 30 sec, elongation at 72°C for 30 sec, and final elongation at

72°C for 5 min. Sanger method sequencing were done to detect T>16297C SNP LEPR gene.

Statistical Analysis

The data were statistically analyzed using the SAS (Statistical Analysis System) program in 2012 (21) to study the effect of leptin receptor gene on a productive and physiological trait of local Iraq chicken. Duncan's analysis 1955 (9) was used to analyze the significance of the discrepancies, and the chi square test was used to examine the percentages of the genotype distribution in the:

Mathematical model: the relationship of genetic phenotypes of the leptin receptor gene (T> 16297C) to the studied traits:

$Yij = \mu + Li + eij$

Since:

Yij : the observation value j of the genotype i

μ : The general average of the trait

Li : Influence of genetic phenotypes of the leptin receptor gene

The symbols in this model are as mentioned in the first mathematical model mentioned above

The Chi-square-$\chi 2$ test was also used to compare the percentages of the distribution of genetic phenotypes for each gene in the studied sample.

$$P_T = \frac{2 * \text{No. of Homozygous} + 1 * \text{No. of Heterozygous}}{2 * \text{Total number of samples}}$$

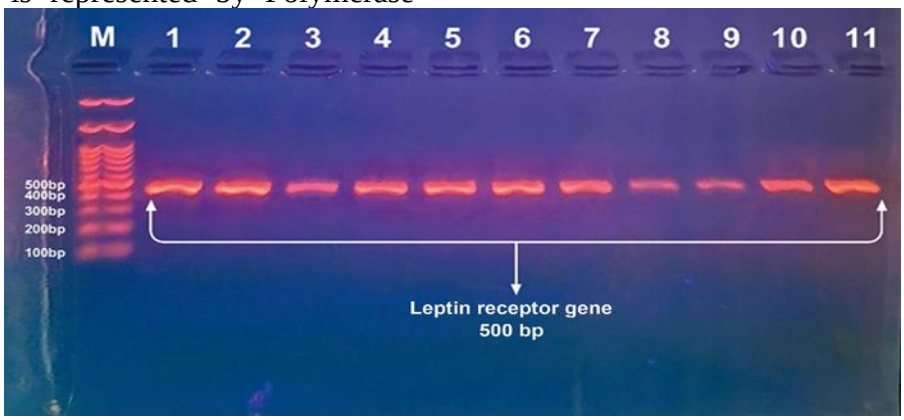
Allele frequency for T allele

Repeat of the first allele: P_T

Since: P + q = 1 then the frequency of the second allele is: $q_C = 1 - P$

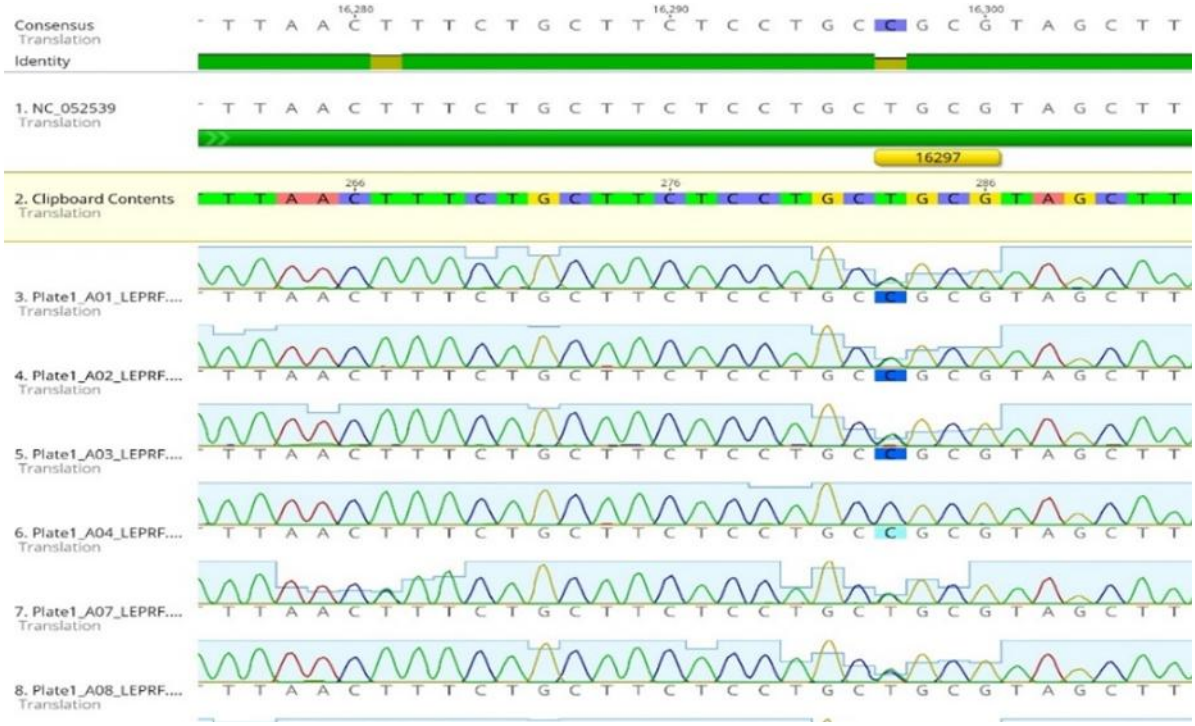
RESULTS AND DISCUSSION

Amplification of the target region of the leptin receptor gene: The fifth intron of leptin receptor gene is represented by Polymerase Chain Reaction (PCR) amplified region with a molecular weight of 500 bp, according to Lei (16). DNA ladder (100-1500 bp) was employed to detect the PCR product, and a digital camera was utilized to take photos of the gel (Figure 1). Lei (16) acquired the same PCR product size.



Finger 1. PCR product of Leptin receptor gene with a molecular weight of 500 bp were electrophoresed on 1.5% Agarose gel at 5 volts/cm^2. After staining with Ethidium Bromide Lane (M) DNA ladder (100-1500), Lane (1-11) PCR products of the leptin receptor was visible under ultraviolet light

Analysis of the nitrogenous bases sequence results of Leptin receptor gene: The results of the PCR product analysis of the genotypes under study TT (wild), TC (heterozygous), and CC (Mutant) it was found that there is a single nitrogen base substitution at site T > 16297 C. The target region size 500 bp of the LEPR gene was determined using the PCR technique within a piece of known size DNA marker of (100-1500) bp in the first hole of the gel, and different genotypes were discovered using the Sanger method sequencing to detect T>16297C SNP were that the T allele Frequency is 0.26 and the C allele Frequency 0.74. However, the proportions differed from previous studies, and this difference may be due to the type of chicken, environmental conditions, or random mating. Furthermore, the allelic frequency and distribution are affected by the random mating of local chickens. It was representing that the wild TT genotype 3%, the heterozygous TC 46% and Mutant CC 51%.(Figures 2)



Figures 2. Alignment of nucleotides sequence analysis of target region with the wild genotype at the NCBI

The result of Table 1 shows high significant ($p<0.01$) differences between the various genotypes of leptin receptor gene, but the mutant genotype CC had the higher percentage amounted 51% followed by the hetero azygous TC amounted 46% then the wild TT genotype 3%, and the mutant C allele had high significant ($p<0.01$) as compared to the T allele.

Table 1. Number and percentage of the genotypes distribution of leptin receptor gene in local Iraqi chickens

SNPs	Genotypes	NO.	Percentage (%)	Allele	Frequency
LEPR16297	TT	3	3	T	0.26
	TC	46	46		
	CC	51	51	C	0.74
	Total	100	100		
	chi-square value (χ^2)	--	**46.72	--	--

**: High significant ($p<0.01$)

The table 2 showed significant differences ($p<0.05$) between CC and TC genotypes in feed intake amounted (1189.23 and 1130.27) gm respectively, followed by TT genotype 925.99 gm in period 1. Also had been observed significant differences ($p<0.05$) in feed intake of CC genotype amounted 1210.16 gm followed by TC genotype amounted 1159.72 gm then genotype TT 998.21 gm in period 2. But there were non-significant between TT and TC genotypes. On other hand it had been observed non-significant differences between the various genotypes of Leptin receptor gene TT, TC and CC on feed intake in period 3, 4, 5 ,6 and 100 days. And there were significant differences ($p<0.05$) between CC and TC genotypes amounted (1858.22 and 1787.13) gm respectively, followed by TT genotype amounted 1580.27 mg in period 7(16 days), and not significant differences between TT and TC genotypes. Denbow *et al.,* (8) referred the central injections of human leptin were related to a decrease in chicken food intake, showing that receptor activity in birds is preserved. The results indicate that leptin does not control energy balance in birds as an adipose tissue-derived signal. Endogenous leptin is not thought to play a significant role in appetite regulation in birds, and leptin and its receptors are relatively low in the hypothalamus, which is responsible for controlling feeding behavior in the biggest section of the brain. (12,14).

Table 2. Effect of LEPR gene polymorphisms (T> 16297C) on feed intake of Local Iraq chicken

(Feed Intake) mean ± std error				Significance level
Period	TT	TC	CC	
1	925.99 ± 17.62 b	1130.27 ± 30.54 a	1189.23 ± 27.89 a	*
2	998.21 ± 37.19 b	1159.72 ± 29.09 ab	1210.16 ± 26.05 a	*
3	1053.27 ± 49.83 a	1186.99 ± 27.57 a	1232.45 ± 24.79 a	N.S
4	1107.81 ± 58.42 a	1218.64 ± 25.34 a	1255.98 ± 22.67 a	N.S
5	1181.42 ± 70.65 a	1251.61 ± 23.02 a	1291.28 ± 19.42 a	N.S
6	1255.35 ± 82.69 a	1286.61 ± 20.37 a	1314.96 ± 17.09 a	N.S
7 (16 days)	1580.27 ± 59.46 b	1787.13 ± 37.51 ab	1858.22 ± 33.78 a	*
100 days	8102.31 ± 375.64 a	9020.95 ± 189.68 a	9352.28 ± 167.73 a	N.S

The means with different superscripts of each row are significantly different.
***: Significant (p<0.05), N.S: Non-significant**

Table 3 referred that were non-significant effect of leptin receptor gene TT, TC and CC genotypes on egg weight in all periods of study.

Table 3. Effect of LEPR gene polymorphisms (T> 16297C) on Egg weight of Local Iraq chicken

(Egg Wight) mean ± std error				Significance level
Period	TT	TC	CC	
1	41.29 ± 1.81	43.37 ± 0.76	42.12 ± 0.52	N.S
2	42.46 ± 1.58	45.41 ± 0.71	44.83 ± 0.54	N.S
3	44.82 ± 2.70	46.30 ± 0.59	46.47 ± 0.50	N.S
4	49.82 ± 3.13	47.89 ± 0.69	48.50 ± 0.64	N.S
5	47.82 ± 1.59	46.06± 0.78	47.30 ± 0.76	N.S
6	47.75 ± 0.87	48.03 ± 0.69	46.14 ± 0.78	N.S
7 (16 days)	45.91 ± 1.74	46.24 ± 0.55	45.23 ± 0.54	N.S
100 days	45.69 ± 1.83	46.19 ± 0.51	45.80 ± 0.43	N.S

The means with same superscripts of each row are non-significantly different
N.S: Non-significant

Table 4 showed there were non-significant differences between TT, TC and CC genotypes of Leptin receptor gene on egg number in all periods of study.

Table 4. Effect of LEPR gene polymorphisms (T> 16297C) on Eggs Number of Local Iraq chicken

(Egg NO.) mean ± std error				Significance level
Period	TT	TC	CC	
1	7.33 ± 1.45	8.98 ± 0.29	8.94 ± 0.34	N.S
2	9.67 ± 1.76	9.70 ± 0.32	9.41 ± 0.34	N.S
3	10.67 ± 1.33	9.74 ± 0.26	9.43 ± 0.34	N.S
4	10.33 ± 0.67	10.09 ± 0.29	9.78 ± 0.31	N.S
5	10.33 ± 0.67	9.80 ± 0.25	9.65 ± 0.28	N.S
6	10.67 ± 1.45	9.71 ± 0.26	9.67 ± 0.26	N.S
7 (16 days)	11.67 ± 1.86	11.17 ± 0.32	11.27 ± 0.29	N.S
100 days	70.67 ± 8.11	69.20 ± 1.30	68.16 ± 1.61	N.S

The means with same superscripts of each row are non-significantly different
N.S: Non-significant

Table 5 shows the CC and TC genotypes had significant (p<0.05) effect on HDL level in blood serum amounted (52.20 and 51.85) mg/dl respectively, followed by TT genotype 36 mg/dl. While in blood serum albumin were high significant differences (p<0.01) effect between CC and TC genotypes amounted (2.44 and 2.39) g/dl respectively, followed by TT genotype amounted 1.97 g/dl. While there were non-significant differences between TT, TC and CC genotypes of Leptin receptor gene in (Glucose, cholesterol, triglyceride, LDL, VLDL, Total protein and globulin).

Table 5. Effect of LEPR gene polymorphisms (T > 16297C) on physiological traits of Local Iraq chicken

(Physiological traits) mean ± std error				Significance level
Traits	TT	TC	CC	
Glucose (mg/dl)	238.00 ± 5.69 a	239.91 ± 3.98 a	237.67 ± 4.78 a	N.S
Cholesterol (mg/dl)	137.67 ± 22.88 a	159.04 ± 6.40 a	164.04 ± 7.12 a	N.S
Triglyceride (mg/dI)	557.33 ± 9.33 a	547.80 ± 4.93 a	544.75 ± 5.41 a	N.S
HDL (mg/dI)	36.00 ± 12.50 b	51.85 ± 1.68 a	52.20 ± 1.41 a	*
LDL (mg/dI)	17.67 ± 2.60 a	22.39 ± 1.07 a	23.78 ± 1.49 a	N.S
VLDL (mg/dI)	84.00 ± 32.19 a	84.80 ± 5.29 a	88.06 ± 5.84 a	N.S
Albumin (g/dl)	1.97 ± 0.38 b	2.39 ± 0.03 a	2.44 ± 0.03 a	**
Total Protein (g/dl)	5.18 ± 0.05 a	5.30 ± 0.09 a	5.41 ± 0.09 a	N.S
Globulin (g/dl)	3.21 ± 0.44 a	2.92 ± 0.08 a	3.00 ± 0.08 a	N.S

The means with different superscripts of each row are significantly different.
***: Significant (p<0.05), **: High significant (p<0.01), N.S: Non-significant**

The table 6 showed there were non-significant differences between the TT, TC and CC genotypes of Leptin receptor gene in Egg Quantitative Trait. In other study Eleven common differentially expressed proteins were discovered in the egg white proteomes of all six species, while numerous genes and proteins were discovered to be uncommon in either the egg proteome or the oviduct proteome of the species (23).

Table 6. Effect of LEPR gene polymorphisms (T > 16297C) on egg quantitative trait of local Iraq chicken

(Egg Qualitative Traits) mean ± std error				Significant level
Egg Quantitative trait	TT	TC	CC	
Egg Shell weight (gm)	7.04 ± 0.20	6.89 ± 0.06	6.98 ± 0.06	N.S
Thickness Egg Shell(mm)	0.40 ± 0.01	0.38 ± 0.00	0.38 ± 0.00	N.S
Yellow Weight(gm)	16.23 ± 0.30	16.16 ± 0.14	16.21 ± 0.13	N.S
Yellow High(mm)	18.56 ± 0.28	18.38 ± 0.11	18.65 ± 0.09	N.S
Yellow Dimeter(mm)	38.73 ± 0.49	38.81 ± 0.14	38.93 ± 0.14	N.S
Albumin weight (gm)	26.05 ± 0.72	26.60 ± 0.30	27.06 ± 0.28	N.S
Albumin Dimeter(mm)	73.75 ± 0.89	74.76 ± 0.49	75.39 ± 0.53	N.S
Albumin High(mm)	6.83 ± 0.32	6.67 ± 0.08	6.82 ± 0.08	N.S

The means with same superscripts of each row are non-significantly different
N.S: Non-significant

Table 7 showed non-significant differences between TT, TC and CC genotypes of Leptin receptor gene on Egg Mass

Table 7. Effect of LEPR gene polymorphisms (T> 16297C) on egg Mass of local Iraq chicken

(MASS Egg) mean ± std error				Significance level
Period	TT	TC	CC	
1	302.90 ± 64.07	389.65 ± 14.69	376.83 ± 15.29	N.S
2	415.12 ± 9076	439.63 ± 15.79	421.37 ± 16.05	N.S
3	475.29 ± 58.45	450.03 ± 12.91	437.65 ± 16.11	N.S
4	518.92 ± 63.23	481.74 ± 14.85	474.42 ± 16.30	N.S
5	493.48 ± 31.73	450.63 ± 13.36	454.11 ± 14.03	N.S
6	507.60 ± 64.55	466.81 ± 13.89	445.91 ± 14.46	N.S
7 (16 days)	534.08 ± 85.27	516.98 ± 16.29	511.12 ± 15.29	N.S
100 days	3231.41 ± 416.57	3191.26 ± 64.97	3121.31 ± 80.65	N.S

The means with same superscripts of each row are non-significantly different
N.S: Non-significant

Table 8 showed that the genotypes were non-significant effect for TT, TC and CC genotype in feed conversion ratio of LEPT gene in all period. There were non-significant effects in the egg quantitative trait and mass egg even in the feed conversion ratio (FCR) of local Iraqi chicken. It may have an effect in previous studies, but we did not get an effect, perhaps

due to environmental conditions, the type of chicken or random mating

Table 8. Effect of LEPR gene polymorphisms (T> 16297C) on feed conversion ratio (FCR) of Local Iraq chicken

(FCR) mean ± std error				Significant level
Period	TT	TC	CC	
1	3.32 ± 0.63	3.12 ± 0.15	3.55 ± 0.24	N.S
2	2.62 ± 0.53	2.84 ± 0.15	3.59 ± 0.58	N.S
3	2.29 ± 0.33	2.77 ± 0.12	3.12 ± 0.19	N.S
4	2.18 ± 0.17	2.69 ± 0.13	3.03 ± 0.28	N.S
5	2.42 ± 0.23	2.89 ± 0.10	3.04 ± 0.15	N.S
6	2.56 ± 0.42	2.89 ± 0.11	3.14 ± 0.13	N.S
7 (16 days)	3.14 ± 0.61	3.71 ± 0.22	3.86 ± 0.18	N.S
100 das	2.65 ± 0.38	2.99 ± 0.10	3.33 ± 0.17	N.S

The means with same superscripts of each row are non-significantly different
N.S: Non-significant

The table 9 was presented significant increase ($p<0.05$) in CC and TC genotypes on age maturity amounted (163.24 and 160.52) days respectively, followed by TT genotype amounted 143.00 day. But there were non-significant differences between TT, TC and CC genotypes of Leptin receptor gene in weight sexual maturity. Ashwell *et. al.*, (3) indicated that LEPR expression changes with age in chicken adipose tissue. The function and value of chicken adipose tissue LEPR are unknown because leptin gene expression in adipose tissue was not responsive to a number of hormones.

Table 9. Effect of LEPR gene polymorphisms (T> 16297C) on age and weight at sexual maturity of Local Iraq chicken

(Age and weight at sexual maturity) mean ± std error				Significance level
Variable	TT	TC	CC	
Age	143.00 ± 7.77 b	160.52 ± 2.78 ab	163.24 ± 2.62 a	*
Weight	1414.00 ± 102.46 a	1318.02 ± 27.00 a	1403.69 ± 33.13 a	N.S

The means with different superscripts of each row are significantly different
***: Significant ($p<0.05$), N.S: Non-significant**

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