Plant Archives Vol. 20 No. 1, 2020 pp. 489-494 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF MUTATION (C33379782T) IN QUANTITATIVE TRAITS LOCI (QTL) IN LOCAL IRAQI CHICKENS ON PRODUCTIVE AND PHYSIOLOGICAL PERFORMANCE

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Abstract

In this study, the local Iraqi chickens were used to detect some genetic changes in some QTL region (C33379782T) and its effect on productive and physiological performance of local Iraqi chickens. PCR-RFLP technology was used to detect this mutation using the restriction enzyme (*Alu1*). C and T Alleles were obtained as wild C allele and mutant T allele. high significant differences ($P < 0.01$) in the percentage of allelic frequency as C allele over T allele It was observed that no significant differences were shown between C and T allele in (sexual maturity age, sexual maturity weight, mean feed consumption, egg mass). the number of egg allele T had significantly effects ($p < 0.05$) in egg production at 2, 6, 100 days productive periods and C had allele significant effect ($p < 0.05$) in the conversion factor in the fifth production period. in mean egg weight T allele had significantly effects ($p < 0.05$) over C allele in productive periods 3, 5, 6. In egg quality, significant differences ($p < 0.05$) were observed in yolk weight and egg white diameter as T allele exceeded C. There were no differences in (egg shell weight, shell thickness, yolk diameter, yolk height, egg white weight, egg white diameter). In physiological characteristics there was significant ($P < 0.05$) effect for T allele over C allele in (cholesterol, HDL, VLDL) concentrations . In the concentration of triglycerides, the allele C significantly ($P < 0.05$) exceeded T and there were no significant effects in the concentration of (glucose, LDL, albumin, total protein, globulin. According to the results obtained, the different alleles of QTL can be used as a genetic indicator in the selection programs in local Iraqi chickens.

Key words: *Quantitative Trait Loci (QTL), Genetic markers, Mutation, Chicken*

Introduction

Poultry production is one of the most important bases of economy in many countries of the world, characterized by the rapid turnover of capital and in fulfilling the food needs of humans. Consequently, genetic developing of local chicken started in the second half of the last century worldwide Sorensen (1997). Due to the adaptation of local Iraqi chickens to environmental conditions and resistance to epidemic diseases in addition to tolerance to thermal stress (AL-Hassan and AL-Jebouri 1998), it was necessary to find indirect means of selection based on measuring the performance of birds at an early age through the use of genetic material DNA (Deoxyribo Nucleic Acid) as an elective tool that reduces the generation period and increases the efficiency of the production unit AL-Hilali *et al.,* (2000), several studies conducted on the economic characteristics of poultry showed that many of them are quantitative qualities, which include age at sexual maturity and production Eggs, egg weight, body weight and food conversion etc.. the importance of studying these traits increased especially after knowing the importance of the of QTL (Quantitative Trait Loci) and its role in the selection and genetic improvement programs. Although economic traits have been studied extensively in local chickens, only little information available on molecular aspects, and the development of molecular biology tools have allowed the genetic basis of quantitative traits to be investigated at the molecular level (Lwelamira *et al.,* 2009). QTL sites represent differences at the genetic level and are characterized by providing us with a true representation of the genetic structure at the level of DNA and they are unchanged by the impact of environmental conditions and can be detected in the early stages of growth and development of animals. It is considered a promising for the future because it involves the adoption of a large

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number of animals and compare them and put them forward for analysis, which allows to identify a large number of its own features and study them simultaneously, and also to identify the genes within QTL and for animals that fall within human consumption (Khalil, 2006) and (Ankra-Badu, 2009).

The identification of QTL affecting growth rate, age of sexual maturity and a range of economic characteristics in chickens is of great biological significance based on some QTL data (www.animalgenome.org/cgi-bin/QTLdb, www.thearkdb.org/arkdb) Tuiskula-Haaristo (2002) stated that a number of QTL Local that have a significant effect on sexual maturity are located on the Z chromosome. Sasaki *et al.,* (2004) indicated that the QTL region affecting the sexual maturity age lies between ADLO201 and MCWO241 of the Z chromosome. On these studies, Xu *et al.,* (2011) indicated that these region of the QTL present on the Z chromosome were influential on the age of sexual maturity in domesticated chickens. Due to the absence of a previous study on the role of QTL sites in Iraqi domestic chickens and their relationship to economic characteristics, this study was conducted to investigate the genetic mutations in some sites of quantitative traits QTL and its relationship to the productive and physiological characteristics of local Iraqi chickens.

Materials and methods

This study was conducted the at poultry form of College of Agriculture - University of Baghdad, where 105 chickens aged ten weeks and tagged in legs. They were distributed into individual cages sequentially 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) egg produced by each chicken were collected, numbered and weighed individually, veterinary measures were all carried out according to the program in location of the breeding of laying chickens and herds of local Iraqi chicken. Birds were fed during the duration of the experiment by providing two types of diets and according to the age of the first herd 11-18 Week and second age 19 week to the end of the experiment, the feed was provided to birds by 100 grams per day, divided into two meals in the morning, evening and also provided the breeding hall with lighting system for the duration of the experiment according to *Lohman* program recommended in the manual breeding of laying hens. 3ml of blood from each chicken aged 16 week were collected from wing vein and put blood in tubes containing anticoagulant (EDTA) and kept at a freezing point -20 until the molecular tests were carried out DNA extraction by Geneaid-Kit Company in Taiwan. 20ml of blood instead of 200ml were taken because all chicken blood cells contain nuclei and nucleic acid. 500ml of GSB Buffer cell solution instead of 200 was added due to the high protein content in chicken serum compared to mammals (AL-Khatib, 2015). The DNA purity was measured using NanoDrop spectrophotometer. By (Japan / shimadzu) the electrophoresis was carried out through 1% agarose gel and 5µl Ethidium bromide by mixing 5ml of extracted DNA samples with 1µl of loading dye for half an hour and 100 volts afterwards. The gel was placed in the UV light Trans illuminator (made in Italy). The origin and imaging of the gel was done by a digital camera. We noted the bundles of colored DNA genetic material due to the presence of Ethidium dye hence, USA / Alpha DNA primers were prepared as a powder at different bicomol concentrations.

To detect the mutation (C33379782T) within 400 bp using the restriction enzyme (*Alu1*) the PCR reaction was performed to amplify DNA transcripts in order to locate the quantitative traits of QTL using the diagnostic kit (GoTaq® Green Master Mix) prepared by the American company Promega.

PCR reaction : The PCR reaction was performed in 0.2 ml tubes by mixing master mix reagents in final volume

Reverse primer	Forward primer
5´GGCGTTTTGTG	5´TCTTCGAACAC
TTTTCTTGGCAT3´	ATTACTCACTGA3´

of 25 µl. The amplification was performed in a TECHNE (T-C5000) thermal cycler and the reaction mixture was prepared according to the procedure that suggested by the manufacture company (Promega, USA) using 75-90ng/µl of DNA and 0.8 µl of primers and then complete the PCR reaction volume to 25 µl by distilled water finally reaction mixture vortexes thoroughly. PCR mixture without DNA template was used as a negative control .Thermal cycle with the following profile: initial denaturation at 94°C for 4 minutes, 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds and a final elongation at 72°C for 5 minutes Eight microliters PCR products were digested with 5 units of *Alu1* at 37°C overnight. Restriction pattern was visualized in a 1.5% agarose gel electrophoresis stained with Ethidium bromide.

Blood samples were collected from 105 chickens aged 30 weeks from the brachial vein with a 5ml syringe and placed in tubes with no anticoagulant then the samples were centrifuged by (Beckmam Tj6 made in Ireland) 8000 cycle per minute for 20 minutes. Serum with an automatic blood chemistry analyzer produced by the Polish

company (Chormay) is equipped with Kits reagents to determine the concentration of glucose, total protein, albumin, cholesterol, triglycerides, LDL (Low Density Lipoprotein) and HDL (High Density Lipoprotein).

The production characteristics were studied for age and weight at sexual maturity also, the number of eggs produced, egg weight, egg mass, feed and feed conversion factor were calculated during 100 days of production starting from the date of laying the first egg and divided into seven production periods representing every two weeks of production 1, 2, 3, 4, 5, 6, 7 Egg quality was calculated, which included the thickness and weight of the shell. the weight height and diameter of the yolk and the weight, diameter and height of the egg white. The data were analyzed using the complete random design and the ready-made statistical program SAS. (2012) the chi-square –x 2 test was also used to compare the percentages of alleles to QTL sites for domestic female Iraqi local chickens.

Results and discussion

DNA extraction Fig. 1 illustrates the DNA extraction process that was successful as a first step followed by the PCR interaction of the target region of the QTL.

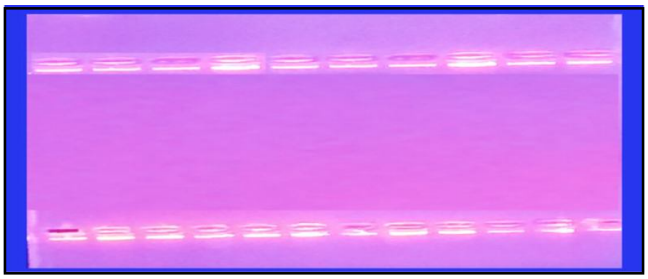

Fig. 1: Electrophoresis of DNA samples extracted from local chicken with electric current 100v for 30 minutes in 1% agarose.

Amplification of QTL by PCR technology using several special tools, starters and samples of DNA then picture the results of the analysis to ensure the success of the extraction process and obtain the target area of QTL length 400bp Fig. 2.

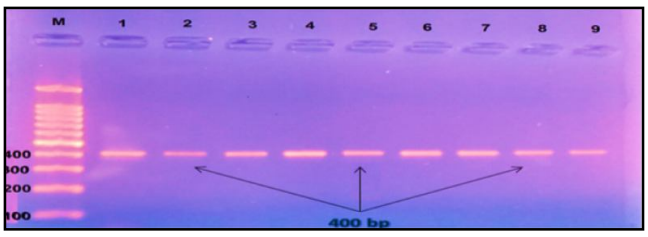

Fig. 2: Electrophoresis of PCR 1.5% agarose, electric current 70v for 90sec, M: Ladder (100-1000) samples (1-9) 400bp.

Alleles were determined for QTL region of Iraqi local chickens using PCR-RFLP technology and restriction enzyme (*Alu1*) as described in materials and methods of work and picture the results of deportation to get the percentage and distribution of alleles in the sample birds as in Fig. 3.

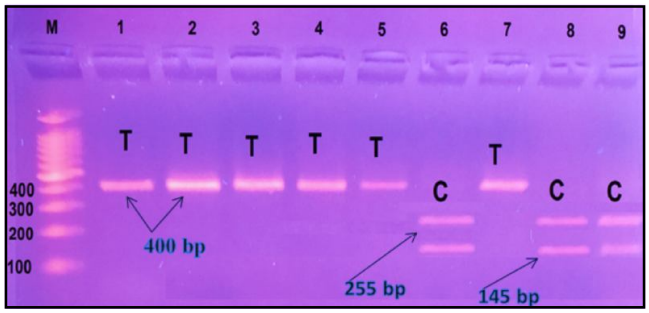

Fig. 3: Result of digesting QTL region with restriction enzyme *Alu1*, 1.5% agarose, Electric current 70v for 90 minutes, M: Ladder (100-1000), Allele T samples (1, 2, 3, 4, 5, 7) 400bp, Allele C (6, 8, 9) (145, 255) bp.

Table 1 shows the distribution and percentages of Iraqi local female chickens showed high significant difference (P<0.01) for C and T Alleles which were 12.38%, 87.62%, respectively as the T mutant allele far clearly passed the C wild allele. The results were close to those by Xu *et al.*, (2011) when using this enzyme and this QTL in local Chinese chickens. The percentage of Chinese chickens was 8.42% and 91.58% for the C and T alleles respectively, the closeness of results may be due to the fact that chicken species have a similar genetic base Lu *et al.*, (2007).

Table 1: Distribution and percentage of QTL Alleles in Iraqi female local chicken.

Percentage	No.	Marker AluI
87.62	92	T Mutant
12.38	13	C Wild
100%	105	Total
62.66**	----	χ^2 Chi-Square
	(P<0.01)**	

Effect of different alleles of QTL on chromosome Z in Iraqi local chickens in both age and body weight at sexual maturity

Considering table 2 there was no significant differences between C and T alleles in the age of sexual maturity as they were in wild C alleles 166.23 days and mutant T 161.51 days. The mean body weight at sexual maturity showed no significant differences between C and T alleles as the wild C was 1448.23 g and the mutant T 1342.79 g the QTL alleles specified in the local Iraqi chickens in the Z chromosome had no significant effects this result was consistent with the results of Xu *et al*

(2011) on the local Chinese chicken also indicated by Soller *et al.* (1984). QTL which is responsible for body weight at sexual maturity are located on several chromosomes 2,4,8,27,Z moreover the detection of one QTL responsible for body weight at maturity sex on chromosomes 1,13 it was also found that the QTL responsible for sexual maturity and the age of sexual maturity combined weight is located on chromosomes 27,13,4,3,1 through the study of hybrid generation that was mixture of two species Ross 308 White leghorn.

Table 2: Effect of different QTL alleles on sexual maturity age and weight.

Age of sexual	C	T	Mean sig. Dif.
maturity(Day)	166.23+6.24a	161.51+1.89a	N.S
Sexual Maturity weight (gm)	1448.23 + 78.65 a	1342.79 + 21.46 a	N.S

Means that carry similar scripts within the same row mutually Non significant

The Relation of different alleles of QTL region on chromosome Z for Iraqi local chickens with some of productive trait :-

Food consumption: Table 3 shows that there was no significant differences in the characteristic of feed consumption between C and T allele within production periods 1, 2, 3, 4, 5, 6, 7 and not within 100 days of production which may be probably due to a standardized diet program. as feed was provided to birds in two parts of the morning and evening by 100 grams per day, to maintain non-increasing weights of birds exceeding the recommended body weight rates during the age in addition to following a light strategy within the recommended programs in breeding laying hens. Perhaps these factors combined reduced the difference in the feed consumption rate between the birds of the experiment.

Egg mass: As Table 3 shows there was no significant differences between C and T alleles in the egg mass characteristic within the different production periods and within 100 days of production, note that this characteristic has not been studied previously, at this site of QTL.

Food conversion factor: Table 3 showed significant differences ($P < 0.05$) between C and T alleles where wild C exceeded and the feed conversion factor 3.39 over the mutant T allele 2.78 during the fifth production period and did not show any significant differences in other periods 1, 2, 3, 4, 6, 7 and 100 days of production this may be due to the absence of significant differences between alleles however the QTL is affected by several genes and sites of quantitative traits and affects them more than they are affected individually.

Number of egg: As shown in Table 4, there were significant differences ($p < 0.05$) between C and T alleles, where the mutant T allele production period 2, 6, 100 days of production which reached 9.25, 9.61, 67.78 egg respectively, while wild C alleles was 8.46, 8.31, 61.575 egg respectively, where there was a significant effect of C and T alleles in the number of egg produced, Khalil *et al.,* (2016) in his study on Egyptian domestic chickens mentioned a number of QTL affecting the weight and the number of egg found on chromosomes 4, Z and C allele reached the age of sexual maturity at 166.23 days, while T the age of maturity was at 161.51 days, resulting in the superiority of T.

Mean of egg weight: The results in table 4 showed significant differences ($P < 0.05$) between C and T alleles, where wild C allele exceeded the mutant T allele in the productive periods 6, 5, 3. C 48.55, 50.39, 53.42 gm, respectively. while T allele 47.18, 48.68, 50.11g, respectively, shows that wild C allele outperformed the mutant T in the mean weight of the egg in more than one productive period to the inverse proportion between the number of egg produced and the weight of the egg which we observed through the superiority of T over C in egg production. Also, the average weight of birds at the sexual

Table 3: Effects of different QTL alleles on food conversion and egg mass and food consumption.

Production period	Food Conversion		Mean Level	Egg mass		Mean Level	Food Consumption		Mean level
	C	T		C	T		C	T	
1	4.10 ± 0.57 a	3.43 ± 0.155 a	N.S	332.28 ± 28.55 a	372.09 ±11.23 a	N.S	1200.62 ±58.10a	1153.73 ±21.38a	N.S
2	3.44 ± 0.37 a	3.23 ± 0.32 a	N.S	400.68 ±77.20 a	433.79 ±14.74 a	N.S	1235.98 ±50.79a	1179.66 ±20.21a	N.S
3	3.22 ± 0.26 a	2.93 ± 0.13 a	N.S	420.82 ±32.52 a	449.16 ±11.14 a	N.S	1266.85 ±43.39a	1206.27 ±19.23a	N.S
4	3.47 ± 0.435 a	2.79 ± 0.15 a	N.S	435.60 ±42.895 a	468.49 ±9.99 a	N.S	1296.95 ±35.27a	1234.91 ±17.65a	N.S
5	3.39 ± 0.49 a	2.78 ± 0.07 b	*	441.90 ±39.32 a	469.81 ±8.09 a	N.S	1330.41 ± 25.96a	1267.00 ±15.84a	N.S
6	3.25 ± 0.23 a	2.84 ± 0.11 a	N.S	443.96 ±30.92 a	481.58 ±9.135 a	N.S	1362.49 ±15.06a	1298.18 ±13.885a	N.S
7	3.14 ± 0.375 a	2.80 ± 0.08 a	N.S	567.43 ±33.96 a	569.65 ±11.445 a	N.S	1569.47 ±12.19a	1502.06 ±13.29a	N.S
100	3.14 ± 0.18 a	2.81 ± 0.07 a	N.S	3042.67 ±186.105a	3244.57 ± 54.88 a	N.S	9262.78 ±234.99a	8841.81 ±117.32a	N.S

Mean which carry different scripts within the same row are mutually significantly different($p<0.05$).

Table 6: Effects of different QTL alleles on Biochemical Blood traits.

Blood traits	C	T	Mean levels
GlucoseMg/dl	242.46 ±3.04 a	229.36 ± 9.32 a	N.S
CholestrolMg/dl	154.66 ±4.57 b	190.71 ±14.14a	*
TriglycerideMg/dl	547.39 ±3.79 a	517.71±27.29 b	*
HDLMg/dl	50.56 ± 1.20 b	58.43±2.39a	*
LDLMg/dl	22.39 ±0.80 a	26.86 ±3.88 a	N.S
VLDLMg/dl	81.70 ±3.92 b	105.43 ±10.22 a	*
Albuming/dl	2.405 ±0.02 a	2.31± 0.08 a	N.S
Total proteing/dl	5.35 ±0.06 a	5.48 ±0.15 a	N.S
Globulin g/dl	2.95 ±0.055 a	3.17 ±0.15 a	N.S

Mean which carry different scripts within the same row are mutually significantly different (p<0.05)

maturity of the C allele was mathematically superior to the T allele, meaning that the average weight of the egg was directly related to the body weight as they increase together (Al Tikriti, 2011). Khalil *et al*., (2016) mentioned that the QTL responsible for egg weight is on chromosome Z.

Relationship of different alleles of quantitative trait loci QTL on the Z chromosome with egg qualities of female local Iraqi chickens

It is noticed from Table 5 that there were significant differences (P < 0.05) between the two alleles T and C in the mean of yolk weight rate and the mean of egg white diameter, where the T allele exceeded the average weight of yolk and reached 16.68 g, while C reached 16.01 g, as far as the characteristic of egg diameter was 74.58 and 77.42 mm for the T and C alleles respectively, and no significant differences were observed for the qualitative characteristics of the egg concerning (weight and thickness of the shell, height and diameter of the yolk, weight and height of the egg white).

Relationship of different alleles of QTL on chromosome Z of Iraqi Local chickens with some biochemical blood traits

Table 6 shows significant differences (P < 0.05) between C and T alleles, where T allele exceeded C in blood cholesterol concentration 190.71, 154.66 mg/dl respectively, note that the normal value of cholesterol in the blood serum birds is Between 100-200 mg / 100 ml, cholesterol levels in blood serum of birds were affected by genetics, nutrition, production period and various diseases (Darraji *et al*., 2008). There were significant differences in the concentration of Triglyceride between the C and T alleles as C outperformed T and reached 547.39 mg/dl and 517.71 mg/dl respectively.

In HDL (high density lipoprotein) levels, T allele significantly (P < 0.05) exceeded 58.43 to C allele 50.56 mg/dl. Also in the concentration of lipoproteins, VLDL density was significantly higher than T allele (P < 0.05) it was 105.43 mg/dl over allele which was 81.70 mg/dl. There were no significant differences between C and T alleles in other blood characteristics, which included the concentration of (glucose, lipoproteins, low density LDL, albumin and protein). Selection based on blood characteristics leads to improvement of the associated productive traits due to high genetic and phenotypic

Table 5: effect of different QTL alleles on egg quality.

Studied features	C	T	Mean level
Mean shell weight	6.88 ±0.04 a	7.03 ±0.11 a	N.S
Mean Shell Thickness	0.38 ±0.00 a	0.38 ±0.00 a	N.S
Mean Yolk weight	16.01 ±0.10 b	16.68 ±0.23 a	*
Mean Yolk height	18.45 ±0.07 a	18.84 ±0.17 a	N.S
Mean Yolk Diameter	38.81 ±0.10 a	38.70 ±0.22 a	N.S
Mean egg white weight	26.53 ±0.21 a	27.41 ±0.53 a	N.S
Mean egg white diameter	74.58 ± 0.37 b	77.42 ±0.83 a	*
Mean egg white height	6.82 ± 0.06 a	6.51 ±0.13 a	N.S

Mean which carry different scripts within the same row are mutually significantly different (p<0.05)

Table 4: Effect of different QTL alleles on egg production and mean egg weight.

Production periods	Mean Egg weight		Mean level	number of eggs		Mean level
	C	T		C	T	
1	42.33 ± 9.675 a	42.33 ± 5.75 a	N.S	7.85 ±0.80 a	8.79 ±0.265 a	N.S
2	47.36 ± 7.20 a	46.90 ± 7.52 a	N.S	8.46±0.895 b	9.25 ±0.30 a	*
3	48.65 ± 8.31 a	47.18 ± 5.70 b	*	8. 65 ±0.815 a	9.52 ±0.26 a	N.S
4	48.83 ± 8.65 a	48.49 ± 5.13 a	N.S	8.92 ±0.85 a	9.66 ±0.275 a	N.S
5	50.39 ± 7.56 a	48.68 ± 4.145 b	*	8.77 ±0.99 a	9.65 ±0.20 a	N.S
6	53.42 ± 5.88 a	50.11 ± 4.67 b	*	8.31 ±0.835 b	9.61 ±0.21 a	*
7	53.455 ± 7.345 a	50.45 ± 5.83 a	N.S	10.615 ±0.73 a	11.29 ±0.21 a	N.S
days 100	49.205 ± 9.97 a	47.73 ± 6.03 a	N.S	61.575±3.84 b	67.78 ±1.15 a	*

Mean which carry different scripts within the same row are mutually significantly different (p<0.05).

correlations between them and the productive traits which are the main goal behind developing chicken genetics. Productivity, which is the main objective of chicken genetic improvement plans (Abbas and Al Suoodi, 2007), this may be due to differences in effect or absence of significant effects due to the presence of these sites of QTL on chromosomes where genes are responsible for the metabolism of fats, proteins and sugars and these sites of QTL may be Part of these genes are effective, they directly or indirectly affect the concentration of proteins, lipids and sugars in the blood plasma(d' Andre' Hirwa *et al.*, 2010).

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