PRESENCE AND ABSENCE OF QUANTITATIVE TRAITS LOCI (QTL) REGION ON CHROMOSOME Z AND ITS EFFECT ON SOME PRODUCTIVE AND PHYSIOLOGICAL TRAITS IN LOCAL IRAQI CHICKEN

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ABSTRACT

This study was carried out to identify the effect of the quantitative trait loci (QTL) region on some productive and physiological traits of Iraqi chickens. One hundred five and ten-week-old laying hens were used and bred in individual sages from sexual maturity to 100 days of production. Blood samples were collected from the brachial vein for DNA extraction and serum isolation, and then polymerase chain reaction (PCR) was done to determine the target region of the QTL. Two groups were obtained: the first had a PCR product with band 294 bp denoted (P) and the second had no PCR product denoted (NP). The P group had a significant ($p < 0.05$) effect on egg weight at the second stage of the study amounted 48.23 gm. and a significant ($p < 0.05$) effect was observed in egg shell weight amounted 6.97 gm. No significant effects were found in all productive, physiological, or qualitative traits.In conclusion, the presence and absence of QTL regions significantly affect egg weight and egg shell weight, which is suitable for selection programs to improve the genetic makeup of Iraqi chicken.

Keywords: PCR, sexual maturity, serum traits ,egg weight, egg shell

مجلة العلوم الزراعية العراقية- 2025 :56 (3):1001-1009 الخطيب وآخرون

وجود وغياب مواقع الصفات الكمية على الكروموسوم Z وتأثيره في بعض الصفات الإنتاجية والفسيولوجية للدجاج المحلي العراقي

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[1 و2] كلية علوم الهندسة الزراعية/ جامعة بغداد [3] التقنيات الدقيقة للثروة الحيوانية والتغذية/كلية جامبلو للتقنيات الزراعية و البايولوجية/ جامعة لييج/ بلجيكا

المستخلص

أجريت هذه الدراسة للتعرف على تأثير منطقة الصفات الكمية (QTL) في بعض الصفات الإنتاجية والفسيولوجية للدجاج العراقي. تم استخدام دجاج بياض بعمر مائة وخمسة وعشرة أسابيع ربيت في اقفاص فردية من النضج الجنسي إلى 100 يوم من الإنتاج. تم جمع عينات الدم من الوريد العضدي لاستخراج الحمض النووي وعزل المصل، ثم تم عمل تفاعل البلمرة المتسلسل (PCR) لتحديد المنطقة المستهدفة منQTL تم الحصول على مجموعتين: الأولى تحتوي على منتج PCR بحزمة 294 زوج قاعدي سميت (P) والثانية لا تحتوي على منتج PCR سميت (NP) كان للمجموعة P تأثير معنوي($P< 0.05$) على وزن البيضة في المرحلة الثانية من الدراسة بلغ 48.23 غم، ولوحظ تأثير معنوي ($P <0.05$) في وزن قشرة البيضة اذ بلغ 6.97 غم، لم يلاحظ تاثِير معنوي في جميع الصفات الإنتاجية، الفسيولوجية والنوعية. استنتج من ذلك ان وجود وغياب مناطق QTL يؤثر معنويا في وزن البيضة ووزن قشرة البيضة، وهو ما يناسب برامج الانتخاب لتحسين التركيب الوراثي للدجاج العراقي.

الكلمات المفتاحية: تفاعل الكوثرة، عمر النضج الجنسي، صفات المصل، وزن البيضة، وزن القشرة.

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INTRODUCTION

Iraqi domestic chickens are one of the important mixed genotypes that must be maintained and preserved because it has many advantages, the most important genetic specifications its adaptation to the prevailing environmental conditions (3.7.14,32) and its resistance to some endemic diseases body composition in broiler (5.11,20) as well as its simple nutritional requirements. Many studies have been conducted on the local chicken, comparing it with the standard strains and crossing between them to improve its production (15.16.18, 23) but it still has less egg and meat yield than the commercial standard strains, which requires further work like special nutrition programs and improve it genetically by unconventional selection (19), which does not consume much effort, time and money, as is the case in traditional methods. Therefore, the need to find modern methods such as searching for technologies that enable the selection of birds based on molecular and DNA-dependent indicators (21). At present, egg hens are selected for many important economic characteristics, which include age at sexual maturity, egg production rate, egg weight, body weight, dietary conversion, shell strength, and albumin height (1,6) , it depends to a large extent on the genotype. According to Mendel's theories, most of them have a genetic base of quantitative type, body weight and other traits they are typical examples of basic quantitative traits and place that affects a certain quantitative trait is called the sites of quantitative traits (QTL) (30.31,34). From the genome, genes that affect the quantitative traits are discovered, which are a sequence of nucleotides that can be traced to the genetic makeup of the animal (25) . Recently the candidate gene are used to improve the productive performance of chicken flocks and large animals a genes (4, 8. 28, 29). Understanding the genetic mechanism between growth rate and age of sexual maturity is of biological importance and it is crucial to determine the QTL affecting productive traits and associated with sexual maturity such as age and weight as it appears. Studies across many animals show that sexual maturity and feeding depends on these traits (6.10.26, 33). Goto and Tsudzuki (13) stated that QTL site analysis is a statistical method for understanding the relationship between phenotype and genetic data regarding egg production traits and egg quality in chickens. More than 890 QTLs have been identified so far and are installed in the QTL database animal (17) The mapping of QTL within chromosomes that contain multiple genetic forms that regulate complex traits is usually followed by the search for mutations causing the observed effects, and this is often a difficult task even after the good mapping process because there are millions of base pairs that contain many genes that need to be investigated and thus to track down the causative mutations. There is a great need for effective bioinformatics strategies such as genes and mutations that regulate growth (2). In view of the absence of previous studies on the sites of quantitative traits QTL in Iraqi domestic chickens, this study was conducted to detect the absence and presence of this target QTL region on Z chromosome and its effect on some physiological traits and productive performance of local Iraqi chickens.

MATERIALS AND METHODS

This study was conducted in the poultry experimental field of the Faculty of Agricultural Engineering Sciences. 105 hens were used at the age of 10 weeks numbered with legs rings, which were raised in individual cages numbered in a series, with the aim of recording egg production from the age of sexual maturity up to 100 days of production. In addition, all vaccinations and preventive measures were carried out according to the recommended programs. Two types of diets were introduced the first was pre-production from 11-18 weeks, and the second rations from 18 weeks until the end of the experiment amounted to 100 g per day. 3 ml of blood from each chicken from the brachial vein and the blood was placed in tubes containing Na-EDTA anticoagulant and kept at a freezing point of – 20 until the molecular tests were carried out and the DNA was extracted by a special extraction kit equipped from the Taiwanese Geneaid company with some important modifications on blood and laysis buffer volume according to Noori (24), after which the target area

amplification was done by specific primers F:TGCAAGCCCAGGAATCATCACTC; R:TAAAACTCTTCTTTCCTTCTACA Xu (32). The target QTL was amplified with the technique of polymerase chain reaction (PCR) using the GoTaq® Green Master Mix diagnostic kit equipped by the American company promega using Thermal Cycler TC-5000 under the following conditions: 94°C for 4 min; 35 cycles of 94°C for 30s, annealing temperature 56°C for 35 s, 72°C for 35 s; and 72°C for 7 min. then the PCR product was carried over using the gel electrophoresis system at an agarose concentration of 1.5% and electric current of 5 volt/ cm after which the amplified DNA bundles were observed under a UV light transilluminator and the amplicon length was determined as 294 pb, Also, blood was collected in the amount of 5 ml from each chicken at the age of 30 weeks and placed in tubes that did not contain an anticoagulant in order to separate the blood serum and to conduct tests on it to find out the concentration of (glucose, total protein, albumin, cholesterol, triglycerides, low-density lipoproteins (LDL), High-density lipoproteins (HDL) and very low-density lipoproteins (VLDL). Furthermore, several productive characteristics were calculated, which are the age of sexual maturity, the weight of sexual maturity, the weight and number of eggs, the mass of egg, the rate of feed consumption, the nutritional conversion factor, and the specific characteristics of the egg. The production period (100) days was divided into seven production periods (1,2,3,4,5,7,8), each period representing two weeks of production.

Statistical analysis: The Statistical computations were done using SAS software program SAS (27) to explore the influence of Genotype of QTL. Duncan's multiple range test to compassion between means. The statistical model was as follows :

$Yij = \mu + Gi + eij$

Yij = dependent variable

μ = overall mean

Gi = effect of QTL groups (P and NP)

eij = error term

RESULTS AND DISCUSSION

DNA extraction and migration and QTL investigation: Figure (1) shows the results of extracting and migrating the DNA samples of local Iraqi chickens. While Figure (2) shows the presence of QTL region and denoted it P and the absence of the amplified segment due to more than one mutation occurring in the same location, and denoted NP.

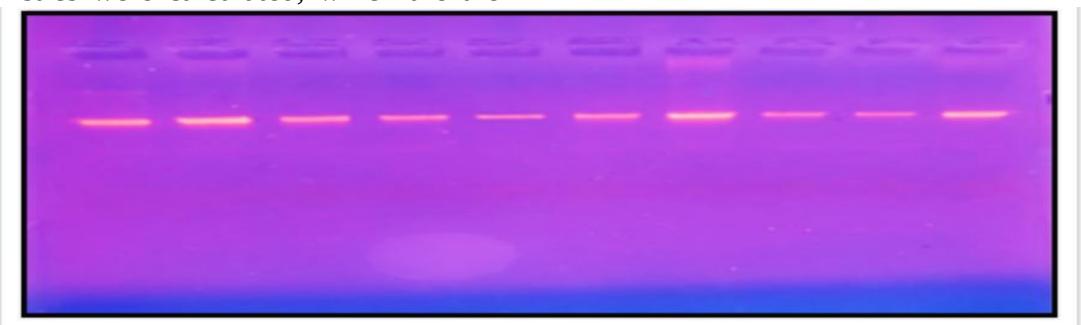

Figure 1. Electrophoresis of DNA samples extracted from local chicken with electric current 100v for 20 minutes in 1% agarose gel

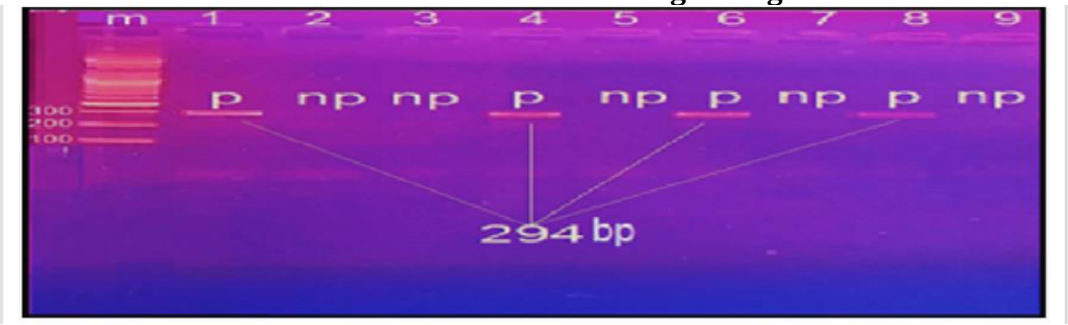

Figure 2. Result of presence and absence of QTL region,1.5% agarose gel, Electric current 70v for 90 minutes, M: Ladder (100-1000), P group samples (1,4,6,8) 294 bp and the NP group (2,3,5,7,9).

Number and percentages of QTL alleles of Iraqi local hens: It is evident from Table(1) that the percentages and the distribution of QTL sites in Iraqi domestic female chickens

showed significant differences (P <0.05) for the two groups P and NP, there was a clear superiority of the wild P allele over the mutant NP allele amounted (61.90 and 38.10) % for each of the P and NP alleles, respectively.

Table 1.Distribution and percentage of QTL alleles of Iraqi local hens

Groups	Number	Percentage (%)
NP (Mutant)	40	38.10
P (Wild)	65	61.90
Total	105	100
Value (χ2)	---	6.50*
	*(P<0.05)	

The effect of the different alleles of the QTL sites on the Z chromosome on the productive traits of Iraqi domestic female chickens

It is clear from Table (2) that the average age of sexual maturity was 160.63 days for the wild P and 164.47 days for the mutant NP. There were no significant differences as for the weight of sexual maturity, it was 1380.06 grams and 1303.50 grams there were no differences in the age of sexual maturity between the alleles within this site 294bp in Chinese chickens (32).

Table 2. Effect of different QTL alleles on age of sexual maturity and weight of sexual maturity (Mean ± SE).

Traits	NP	P	Level of significant
Age of sexual maturity (Day)	164.47+3.08a	160.63+2.25a	N.S
Sexual Maturity weight(gm)	1303.50+ 28.74a	1380.06 +28.93a	N.S

Means that carrying similar scripts within the same row is mutually Non-significant: N.S.no significant

Table (3) shows significant differences in the second production period in the weight of eggs, as P allele was recorded at 48.23 grams, significantly superior to the NP allele 43.59 grams within 100 days of production, there were no significant differences. As for the average number of eggs produced, there were no significant differences between the two alleles, NP and P, in all production periods

Table 3. Effect of different QTL alleles on egg production and mean of egg weight.(Mean ± SE)

Production periods	Egg weight mean(gm)		Level of significance	Number of Egg		Level of significance
	NP	P		NP	P	
1	41.03±0.80^{a}	43.21±5.96a	N.S	9.15±0.34^{a}	8.38±0.35^{a}	N.S
2	43.59±5.24^{b}	48.23±2.56a	*	9.67±0.36^{a}	8.63±0.41^{a}	N.S
3	46.88±2.26^{a}	48.15±1.66a	N.S	9.70±0.39^{a}	9.14±0.33^{a}	N.S
4	49.05±1.63^{a}	49.68±0.92a	N.S	10.00±0.36^{a}	9.31±0.36^{a}	N.S
5	48.62±0.83^{a}	49.30±3.36a	N.S	9.87±0.35^{a}	9.34±0.26^{a}	N.S
6	50.16±2.53^{a}	50.70±4.57a	N.S	9.47±0.33^{a}	9.43±0.28^{a}	N.S
7	49.18±0.77^{a}	50.32±0.56a	N.S	11.40±0.31^{a}	11.09±0.27^{a}	N.S
days 100	47.03±0.80^{a}	49.04±0.91a	N.S	69.27±1.48^{a}	65.32±1.58^{a}	N.S

***: Mean which carry different scripts within the same row are mutually significantly different (p<0.05), N.S.no significant**

The effect on feed conversion factor, egg mass, and average feed consumption. Table (4) shows non-significant differences between the two alleles, NP and P, within the above-mentioned characteristics and in all production periods. Table (5) shows significant differences in the average eggshell weight between the various alleles and no significant differences were observed between the averages of other qualitative characteristics of the egg, represented by (shell thickness, yolk weight, yolk height, yolk diameter, white weight, white diameter, and white height).

Table 4. Effects of different QTL alleles on food conversion, egg mass and food consumption (Mean ± SE).

Production period	Feed Conversion		Level of significance	Egg Mass(gm)		Level of significance	Food Consumption(gm)		Level of significance
	NP	P		NP	P		NP	P	
1	3.47±0.27[a]	3.53±0.18[a]	N.S	375.41±16.33[a]	362.08±13.69[a]	N.S	1164.21±31.85[a]	1156.66±25.93[a]	N.S
2	3.61±0.71[a]	3.03±0.15[a]	N.S	421.51±16.33[a]	442.13±23.58[a]	N.S	1192.64±30.42[a]	1182.93±24.08[a]	N.S
3	2.89±0.18[a]	3.02±0.16[a]	N.S	454.70±16.57[a]	440.10±13.69[a]	N.S	1219.65±28.39[a]	1210.15±22.85[a]	N.S
4	2.59±0.08[a]	3.04±0.22[a]	N.S	490.49±10.60[a]	462.52±15.10[a]	N.S	1247.96±25.78[a]	1239.29±20.84[a]	N.S
5	2.74±0.09[a]	2.93±0.13[a]	N.S	479.88±12.38[a]	460.43±11.43[a]	N.S	1280.22±22.89[a]	1271.55±18.55[a]	N.S
6	2.96±0.22[a]	2.85±0.09[a]	N.S	474.98±14.37[a]	478.11±11.41[a]	N.S	1310.76±19.92[a]	1303.30±16.08[a]	N.S
7	2.80±0.11[a]	2.87±0.11[a]	N.S	560.63±16.37[a]	558.01±14.38[a]	N.S	1507.42±19.58[a]	1512.24±15.15[a]	N.S
100	2.80±0.10[a]	2.88±0.09[a]	N.S	3257.60±73.07[a]	3203.37±73.65[a]	N.S	8922.85±171.65[a]	8876.13±138.55[a]	N.S

Means that carry similar scripts within the same row mutually Non-significant, N.S.no significant

Table 5. Effect of different QTL alleles on egg qualitative traits (Mean ± SE)

Traits	NP	P	Level of significance
shell weight (gm)	6.19±0.06[b]	6.97±0.54[a]	*
shell Thickness (mm)	0.38±0.00[a]	0.38±0.00[a]	N.S
Yolk weight (gm)	16.15±0.15[a]	16.06±0.12[a]	N.S
Yolk height (mm)	18.53±0.10[a]	18.48±0.09[a]	N.S
Yolk Diameter (mm)	38.74±0.15[a]	38.83±0.12[a]	N.S
egg white weight (gm)	26.20±0.35[a]	26.92±0.23[a]	N.S
egg white diameter (mm)	74.80±0.58[a]	75.02±0.43[a]	N.S
egg white height (mm)	6.75±0.09[a]	6.80±0.07[a]	N.S

***: Means which carry different scripts within the same row are mutually significantly different (p<0.05). N.S: no significant**

Results in Table (6) show non-significant differences between the two alleles, NP and P, in the mean concentrations of serum components (glucose, cholesterol, triglycerides, high-density lipoproteins, low-density lipoproteins, very low-density lipoproteins. Blood albumin, total protein, and globulin).

Table 6. Effects of different QTL alleles on biochemical blood raits (Mean ± SE)

Blood serum traits	NP	P	Level of significance
Glucose (mg/dl)	245.97+4.20[a]	237.57+3.92[a]	N.S
Cholesterol (mg/dl)	156.97+6.44[a]	160.78+6.13[a]	N.S
Triglyceride (mg/dl)	544.12+5.54[a]	543.19+7.08[a]	N.S
HDL (mg/dl)	50.63+1.55[a]	52.16+1.53[a]	N.S
LDL (mg/dl)	21.85+1.29[a]	23.66+1.13[a]	N.S
VLDL (mg/dl)	84.49+5.32[a]	84.95+5.07[a]	N.S
Albumin (g/dl)	2.39+0.03[a]	2.39+0.03[a]	N.S
Total protein (g/dl)	5.38+0.10[a]	5.35+0.07[a]	N.S
Globulin (g/dl)	2.99+0.08[a]	2.97+0.07[a]	N.S

Means that carry similar scripts within the same row mutually Non-significant, N.S: no significant

After observing Table (1), which represents the distribution of the QTL loci on chromosome Z in local Iraqi chicken that the wild allele (P) has been superior over the mutant allele (NP), it can be shown that the above result is considered normal because wild genotypes are prevalent in different societies, regardless of the number and type of genetic mutations that occur due to random mating without control. The absence of significant differences with respect to age and weight of sexual maturity between the alleles (P) and (NP) in this gene site is attributed to the types of chickens that may have a similar genetic base Lu (22,1). It is possible that genetic mutations in the P group led to a change or not (silent mutation) in the genetic code (codon) for one or more amino acids, which led to a negative effect on the gene expression of some hormones that affect egg weight and that, are directly related to this QTL region on the Z chromosome. This might probably be the main reason that there is no significant difference between (P) and (NP) alleles with respect to the number and weight of eggs. The fluctuation of significant and insignificant differences between (P) and (NP) alleles in feed conversion index, egg mass and food consumption mean level might be attributed to the environmental and nutritional influence, that directly affects the gene expression of QTL. In general, birds need optimal environmental and nutritional conditions for optimal genetic expression for productive traits. Goto (12) mentioned that QTL affects qualitative characteristics. The external characteristics of the egg are present on the chromosomes (9,14,19), while the effect on the internal characteristics of the egg is within the chromosomes (2, 3, 4, 6, 7, 8, 9, 10, 17, 19). No previous studies are showing the effect of quantitative traits loci on the qualitative traits of the egg. For this reason, no comparison was made with previous researches. All these reasons clarify the absence of significant differences between the internal and external characteristics of the egg. The absence of significant differences between the two alleles (P) and (NP) on some blood biochemical characteristics might be attributed to the fact that this region of the studied QTL has no association with some physiological blood characteristics of chickens within the environmental and nutritional conditions of

this study. The results may differ according to the experimental conditions and the breed of chicken, as well as the selection of other QTLs that may affect these characteristics.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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