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Research Article

Genotyping of TSH-β GENE G>141A associated with some productive and physiological traits of local Iraqi chicken

Mohammad A.K. Al-ANI, Bassam G.M. Al-KHATIB*

Department of Animal Production, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

Email: bassam.ghazi@coagri.uobaghdad.edu.iq

Abstract: This study aimed to detect the SNP G>141A of the TSH-β gene and to discuss their association with some physiological and productive traits in local Iraqi chicken. For this purpose, 100 local Iraqi chickens at 67 days of age were placed in individual cages, and their egg quality measurements were done from 2021 to 2022 to detect the different genotypes. The traits were measured from the sexual maturity up to 100 days for each chicken. The blood samples were collected from 100 chickens at 38 weeks from their brachial vein. PCR REFLP method was used and the PCR product was digested by *MSP1* endonuclease enzyme to detect G141A SNP. Three genotypes were detected viz. GG genotype (wild), GA genotype (heterozygous), and AA genotype (mutant). The G allele frequency was 0.865 compared to the A allele (0.135). There was a significant effect (P<0.05) of the AA genotype of the TSH-β gene on egg mass, followed by the GG genotype and then the GA genotype in period 2. The AA genotype had a significant effect on yellow height, weight, and diameter, and albumin weight, diameter, and height.

Keywords: PCR, MSP1 Enzyme, RFLP-PCR, Maturity.

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Introduction

One of the world's most important economies is poultry production, and the production of table and hatching eggs is one of the most critical aspects of guaranteeing internal and worldwide food security. This production necessitates flocks of highly productive chickens to meet the ever-increasing consumer demand. Poultry farming, which is renowned for its high capital turnover and contribution to satisfying human nutritional needs, is one of the most significant economic pillars in many countries all over the world. Consequently, regional chicken's genetic evolution started in the second half of the 20th century (Sorensen 1997). Finding indirect techniques of selection based on monitoring the performance of young birds using DNA, Thyroid hormones are essential for embryonic and neonatal development in chicks, including hatching. Reduced physiological levels of thyroid hormones inhibit embryonic growth and development. This is visible with methimazole medication, which reduces the bird's thyroid hormone production and growth.

Thyroid hormones are another possible mediator of temperature effects (Caro et al. 2013). It inhibition disturbed prenuptial molt, fattening, mass increase, flight muscle hypertrophy, migratory restlessness expression, and gonadal recrudescence, whereas T4 injection corrected these effects (Pérez et al. 2016, 2018). These findings suggest that thyroid hormones play an important function during the vernal period. TSH stimulates the expression of the biosynthetic enzyme Type II iodothyronine deiodinase in the Medio basal hypothalamus, which directs the conversion of T4 to the biologically active T3 and, in

turn stimulates the release of GnRH from the median eminence to initiate breeding (Hahn et al. 2015; Nishiwaki-Ohkawa & Yoshimura 2016). In Siberian hamsters and domestic chicks, evidence is mounting that hypothalamic T3 may also influence food intake and fatten via the AGRP neurons of the arcuate nucleus (Boswell & Dunn 2017). There were many factors affecting egg quality and quantity, like feeding and breeding (Abdel-Abbas & Al-Marsoumi 2014).

Transthyretin (TTR), which has a high affinity but a low capacity, and albumin, which has a low affinity but a large capacity, are the two principal thyroid hormone distributor proteins in birds. Many mammals' bloods contain the highly high-affinity T4-binding protein thyroxine-binding globulin (TBG), which bird's lack. In addition, unlike mammals, TTR from birds and other no mammalian vertebrates have a higher affinity for T3 than for T4 (Richardson 2014). There were many candidate genes that could be used in selecting programs to improve the genetic makeup of local Iraqi chicken that increasing in egg production, and these candidate genes are TSH-β, VLDLR, estrogen receptor 1, estrogen receptor 2 and FOXL12 genes, and growth hormone (Lei et al. 2007; Al-khatib et al. 2018; Abdulwahid et al. 2019; Abu-Rekaiba et al. 2021a; Rekaiba et al. 2021b). The purpose of this study is to detect the SNP G>141A of the TSH-β 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 the Department of Animal Production, College of Agriculture, University of Baghdad. The produced eggs by each chicken were collected, numbered, and weighed individually, and measurements were done according to the program in the location of the breeding of laying chickens and herds of local Iraqi chickens. This was done to record the production of egg per chicken for 100 days (from sexual maturity to the age of 100 days of production). On the first day on the supplied farm, water with vitamin C 0.5g/l was provided. Throughout the experiment, meals were delivered to the birds at 100g per day and the Lohman lighting system was used during breeding. From each chicken, at 31 weeks, 5ml of blood was collected from its brachial vein, placed in tubes containing an anticoagulant (EDTA), and kept in a freezer (-20^{o}C). DNA extraction was done using the Geneaid-Kit Company (Taiwan). Some modification to the extraction protocol was done by reducing blood value to 20ml (Noori et al. 2019).

The genomic DNA electrophoreses were performed with 1% Agarose gel and 0.2µl Ethidium bromide, then visualized by UV Light, a digital camera was used to get a photo of the gel. The PCR technique condition was carried out to target the region of the TSH-β gene by primers of F: 5-GAGCACGGTGAGCATTACTGG-3 and R: 5-GGAGGTACATTTCTGCCACGT-3.

Using the diagnostic kit (GoTaq® Green Master Mix, Promega) with molecular weight 485bp, the PCR condition was: initial at 94°C for 4 min, then 35 cycles of initial denaturation at 94°C for 30s, annealing at 61°C for 30sec, elongation at 72°C for 30sec, and final elongation at 72°C for 5 min. DNA ladder (100-1500bp) was employed to detect the PCR product, and a digital camera was utilized to take photos of the gel (Fig. 1). *MSPI* enzyme were done to detect G>141A SNP.

Statistical Analysis: The data were analyzed using the SAS (Statistical Analysis System) to study the effect of the TSH-β gene on a productive and physiological trait of local Iraq chicken. Duncan's test was used to analyze the significance of the discrepancies, and the chi-square test to examine the percentages of the genotype distribution.

Model: The relationship of genetic phenotypes of the TSH-β gene (G>141A) was used to the studied trait as follows: $\mathbf{Y_{ij} = \mu + F_i + e_{ij}}$, where, Yij is the observation value j of the genotype i, µ = the general average of the trait, Fi = influence of genetic phenotypes of the TSH-β gene, and eij = the random

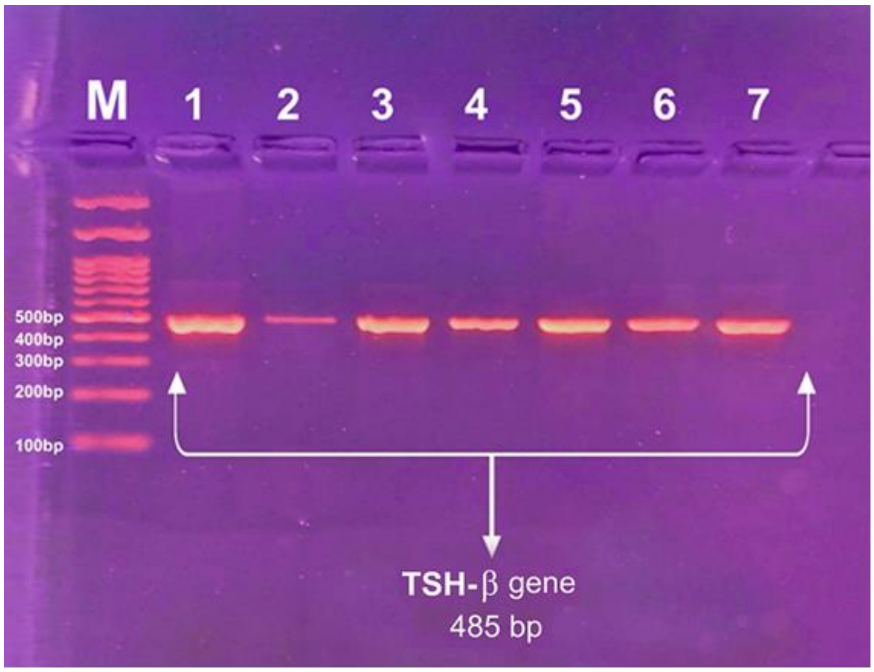

Fig.1. PCR product of TSH-β gene with a molecular weight of 485 bp were electrophoresed on 1.5% Agarose gel at 5 volts/cm^2. After staining with Ethidium Bromide Lane (M) DNA ladder (100-1500), Lane (1-7) PCR products of the TSH-βwas visible under ultraviolet light.

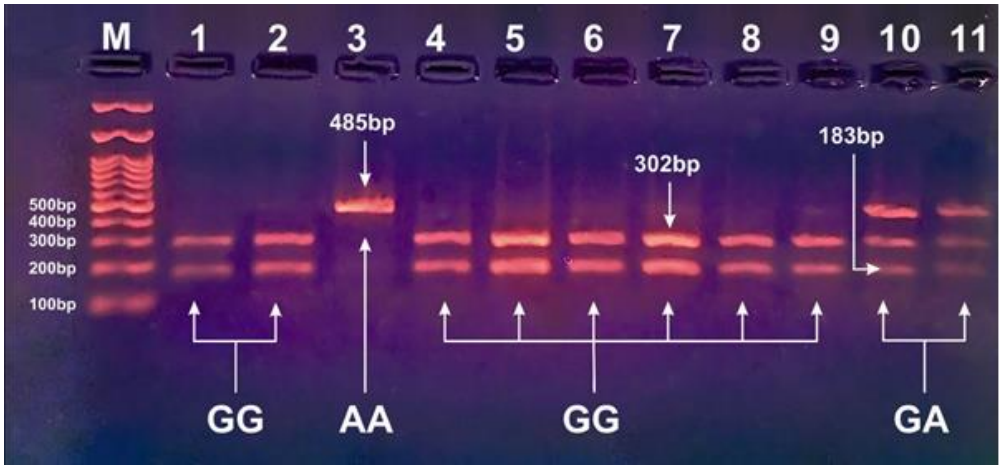

Fig.2. PCR products were digested with *MSPI* electrophoresis on 2%. Lane (M): DNA ladder (100-1500). Lane 1, 2, 4, 5, 6, 7, 8, and 9: wild type GG genotype 302 and 183pb. Lane 10 and 11: heterozygote GA genotype 485, 302 and 183bp. Lane 3: Mutant AA genotype 485bp. The (RFLP) products were agarose gel at 5 volt/cm^2 for 1 hour. Visualized under UV light after staining with ethidium bromide.

error is normally distributed with a mean of zero and a variance of σ2e.

Results and Discussion

Amplification of the target region of the TSH-β gene: PCR amplified region with a molecular weight of 485bp, (Lei et al. 2005). The result was in agreement with the previous study (Lei et al. 2005).

Detection of Enzyme Digestion by Agarose Electrophoresis: The PCR product underwent restriction digestion with *MSP1* enzyme CC/GG to detect SNP G141A of the TSH-β gene and was able to cut the wild genotype G. The following fragment size patterns were observed by 2% agarose gel electrophoresis (Fig. 2). (1) Wild type GG: *MSP1* was cut the sequence to show two fragments in agarose gel electrophoresis (302bp+183bp) (Fig. 2, lanes 1, 2, 4, 5, 6, 7, 8 and 9), (2) heterozygous type

Table 1. Number and percentage of the genotype's distribution of the TSH- β gene in local Iraqi chickens.

TSH-β	Genotypes	NO.	Percentage (%)	Allele	Frequency
TSH-β	GG	76	76	G	0.865
	GA	21	21		
	AA	3	3	A	0.135
	Total	100	100		
	chi-square value (χ^2)	--	**140.22	--	--

**: High significant (P<0.01)

Table 2. Effect of the TSH-β gene polymorphisms (G>141A) on feed intake of local Iraq chicken.

Period	Feed intake (Mean±SE)			Significant level
	GG	GA	AA	
1	1159.61±23.90	1135.96±43.69	1145.14±124.53	N.S
2	1184.91±22.25	1164.81±42.37	1181.99±109.85	N.S
3	1209.60±21.18	1191.24±39.83	1223.52±90.03	N.S
4	1238.31±19.43	1216.43±36.13	1259.70±73.25	N.S
5	1272.08±17.36	1256.62±31.24	1302.17±50.95	N.S
6	1301.02±15.40	1292.35±27.05	1331.92±33.71	N.S
7 (16 days)	1822.53±29.15	1797.13±52.93	1821.99±133.79	N.S
100 days	9188.06±145.55	9054.54±266.68	9266.43±611.43	N.S

Table 3. Effect of the TSH-β gene polymorphisms (G>141A) on egg weight of local Iraqi chicken.

Period	Egg weight (Mean±SE)			Significant level
	GG	GA	AA	
1	43.34±0.50	40.21±0.90	42.98±1.86	N.S
2	45.31±0.46	43.99±1.15	45.14±0.74	N.S
3	46.53±0.40	45.56±1.05	46.92±1.33	N.S
4	48.41±0.52	47.51±1.13	49.68±0.52	N.S
5	46.63±0.62	46.89±1.14	48.77±1.34	N.S
6	46.63±0.55	48.14±1.42	50.22±2.01	N.S
7 (16 days)	45.94±0.40	44.60±1.04	47.85±1.48	N.S
100 days	46.11±0.35	45.27±0.89	47.36±0.45	N.S

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

GA: *MSP1* was cut the sequence to show three fragments in agarose gel electrophoresis. (485bp+302bp+183bp) (Figs. 1-2, lanes 10 and 11), and (3) Mutant homozygote type AA: No cleavage of the whole 485bp segment *MSP1* (Fig. 2, lane 3).

The findings of the current study showed significant differences (P<0.01) between the various genotypes of the TSH-β gene frequency; i.e. the wild genotype GG had higher frequency (76%) followed by the hetero azygous GA (21%) and AA (3%) (Table1). The wild type showed a significant frequency (P<0.01) compared to the mutant allele A (Table 1). In addition, no differences were observed between the TSH-β gene genotypes GG, GA and AA on feed intake during the study period (Table 2). Non-significant differences were found between the various genotypes GG, GA, and AA of the TSH- β gene in the egg weight in all periods (Table 3). The chickens with single or several injections of T3 reduce egg laying rate, ovarian weight, and plasma LH and sex steroid concentrations in a dose-dependent manner (Sechman et al. 2009).

Table 4. Effect of TSH-β gene polymorphisms (G > 141A) on the number of eggs of Local Iraqi chicken.

Period	Egg NO. (Mean±SE)			Significant level
	GG	GA	AA	
1	8.93±0.25	8.81±0.55	9.00 ± 1.15	N.S
2	9.55±0.25	9.24±0.62	11.67±0.33	N.S
3	9.62±0.23	9.57± 0.58	9.67±0.67	N.S
4	9.89±0.23	10.19±0.50	9.33±0.88	N.S
5	9.66±0.21	9.90±0.40	10.67±0.88	N.S
6	9.61±0.21	10.14±0.42	9.67±0.88	N.S
7 (16 days)	11.20±0.24	11.38±0.51	11.33±0.88	N.S
100 days	68.46±1.13	69.24±2.80	71.33±2.73	N.S

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

Table 5. Effect of the TSH-β gene polymorphisms (G>141A) on physiological traits of local Iraqi chicken.

Traits	Physiological traits (mean±SE)			Significant level
	GG	GA	AA	
Glucose (mg/dl)	236.11±3.76	249.29±4.06	230.33±10.11	N.S
Cholesterol (mg/dl)	162.34±5.37	154.76±10.76	196.00±29.57	N.S
Triglyceride (mg/dI)	543.97±4.55	554.62±3.62	554.67±6.74	N.S
HDL (mg/dI)	52.25±1.17	48.33±3.12	56.33±7.45	N.S
LDL (mg/dI)	23.95±1.11	20.19±1.43	17.33±2.40	N.S
VLDL (mg/dI)	86.14±4.35	86.24±9.82	95.33±21.99	N.S
Albumin (g/dl)	2.39±0.02	2.41±0.08	2.44±0.06	N.S
Total Protein (g/dl)	5.35±0.07	5.39±0.09	5.39±0.08	N.S
Globulin (g/dl)	2.95±0.06	3.03±0.10	2.95±0.13	N.S

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

The results showed no significant differences between the various genotypes (GG, GA, and AA) of the TSH-β in the number of eggs (Table 4). Non-significant differences were recorded between genotypes GG, GA, and AA of the TSH-β in serum blood of physiological traits (Table 5). Climate and environmental effects can influence physiological traits, but we found no effect on the examined traits in our study.

A highly significant increase (P<0.01) was observed in the mean of yellow height in AA and GG genotypes (19.17 and 18.60g, respectively), followed by the GA genotype (18.15g) (Table 6). There was a non-significant effect in the mean of the yellow height of GG and GA genotypes. There was a highly significant increase (P<0.01) of the AA genotype in the means of yellow weight, and diameter, albumin weight and diameter (16.85g, 40.48mm, 29.43g, and 71.29mm, respectively), followed by GG and GA genotypes. There were no significant differences between GG and GA genotypes in the means of yellow weight, yellow diameter, albumin weight and diameter. The mean of albumin height significantly increased (P<0.05) in the AA genotype (7.58mm), followed by GA and GG (6.77 and 6.72mm, respectively). The various genotypes of the TSH-β gene had no differences in egg quantitative traits i.e. egg shell weight and thickness. Burch & Lebovitz (1982) pointed out that growing chicks can be mediated by increases in circulatory IGF1 concentrations or by direct effects of T3 via thyroid hormones' stimulatory action on growing tissues. Thyroid in vitro stimulates the growth of chicken embryo cartilage. (Burch & Van Wyk 1987)

Table 6. Effect of the TSH-β gene polymorphism (G>141A) on quality characteristics of the eggs of local Iraq chicken.

Traits	Egg quantitative trait (mean±SE)			Significant level
	GG	GA	AA	
Egg Shell weight (g)	6.94±0.05a	6.90±0.11a	7.23±0.10a	N.S
Thickness of egg shell (mm)	0.38±0.00a	0.39±0.00a	0.40±0.01a	N.S
Yellow weight (g)	16.39 ± 0.10a	15.39±0.21b	16.85±0.68a	**
Yellow height (mm)	18.60±0.08ab	18.15±0.16b	19.17±0.33a	**
Yellow dimeter (mm)	38.90±0.11b	38.55±0.23b	40.48±0.67a	**
Albumin weight (g)	27.04±0.23b	25.65±0.40b	29.43±1.67a	**
Albumin dimeter (mm)	76.11±0.39b	71.74±0.74 b	71.29±1.63a	**
Albumin height (mm)	6.72±0.06b	6.77±0.13 b	7.58±0.50a	*

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

Table 7. Effect of the TSH-β gene polymorphisms (G > 141A) on mass eggs of local Iraq chicken.

Period	Mass egg (mean±SE)			Significant level
	GG	GA	AA	
1	387.22±11.85a	355.54±24.88a	385.53±45.68a	N.S
2	432.43±12.04ab	405.44±29.42b	526.25±11.69a	*
3	447.15±11.09a	433.40±27.63a	454.27±38.63a	N.S
4	479.47±12.71a	480.21±23.55a	462.77±38.79a	N.S
5	448.78±11.04a	461.72±19.15a	521.77±52.41a	N.S
6	447.97±10.98a	487.91±24.74a	481.99±26.23a	N.S
7 (16 days)	515.02±12.37a	508.96±27.18a	540.30±31.21a	N.S
100 days	3155.13±55.87a	3131.41±142.13a	3376.54±104.02a	N.S

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

Table 8. Effect of TSH-β gene polymorphisms (G>141A) on feed conversion ratio (FCR) of local Iraqi chicken.

Period	FCR (mean±SE)			Significant level
	GG	GA	AA	
1	3.25±0.14	3.70±0.47	3.13±0.68	N.S
2	2.95±0.13	2.29±1.38	2.25±0.22	N.S
3	2.88±0.11	3.14±0.37	2.72±0.24	N.S
4	2.87±0.19	2.78±0.31	2.73±0.08	N.S
5	2.99±0.10	2.88±0.22	2.55±0.30	N.S
6	3.06±0.10	2.83±0.20	2.78±0.18	N.S
7 (16 days)	3.77±0.16	3.83±0.33	3.38±0.21	N.S
100 das	3.11±0.09	3.35±0.35	2.79±0.20	N.S

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

Based on the results a significant effect (P<0.05) of the AA genotype on egg mass was found which was 526.25g, followed by the GA genotype (405.44mg) (Table 7). There were non-significant differences between GG, GA, and AA genotypes in egg mass. Abdalhag et al. (2015) showed that SNPs are linked to growth in candidate genes involved in the insulin and T3 signaling systems. The result also

showed insignificant differences between the various GG, GA, and AA genotypes of the TSH-β gene in feed Conversion Ratio (FCR) during the experiment (Table 8). Thyroid hormone (TH) is essential for growth, development, and protein, lipid, and carbohydrate metabolism (Porterfield & White 2007).

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