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Association of *DIO2* Gene Polymorphism with Some Productive Traits and Prolificacy in Local Awassi Sheep

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Abstract: In this study 40 ewes of native Awassi breed and their offspring of 46 birth which belong to Department of Animal Production/College of Agriculture / University of Baghdad for the period of January 2018 to July 2018. The aim of the study was to determine the polymorphism distribution and allele frequency of *DIO2* (exon 2) gene and its relationship with the productive traits and prolificacy. In PCR analysis, specific primer was used to amplify 350bp fragment flanking the polymorphism site Restriction fragment length Polymorphism (RFLP) applied on PCR Product by using (BstNI) restriction enzyme and genetic varied manifestations (Polymorphism) to the target area of the gene encoding *DIO2* (exon 2) depending on the different enzymatic digestion resulting, CC wild, CG and GG, it amounted to distribute genetic manifestations of gene *DIO2* ratios in a sample sheep Awassi thoughtful 82.50, 12.50 and 5.00% for each of the genotypes and it was the contrast between these highly significant proportions ($P<0.01$), allele frequency were 0.89 and 0.11 of each allele, G and C respectively. Total milk production significantly affected($P<0.05$) by *DIO2* gene polymorphism, the highest production was in ewes with CG genotype (82.20± 4.21 kg), but non-significant effect in lactation period. Percentage of milk contents, fat, protein and sold non-fat affected by this *DIO2* gene polymorphism ($P<0.05$), and non significant in milk lactose percentage. Effect of genotype of LPR gene in prolificacy average was significant ($P<0.05$) for the Awaasi sheep that had the CG (1.20 ± 0.05 lamb/ewe) and CC (1.15 ± 0.06 lamb/ewe) genotype in this study. Concluded through the study of the genotype of *DIO2* gene and its future it adoption to set the genetic rehabilitation strategies for sheep to maximize the economic return of farmed elect and crossing genotypes that have achieved the milk production and best prolificacy projects.

Keywords: Local Awassi sheep, *DIO2* gene, Milk production, Prolificacy.

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Introduction:

There are three main breeds of sheep in Iraq which are the Awassi, the Karadi and the Arabi sheep. Awassi sheep mainly distributed in Iraq, southern Turkey Palestine, and Jordan. It represent about 60% of all sheep in Iraq, while Karadi sheep are mainly found in the north of Iraq. Awassi sheep has high ability to tolerate the hard environmental condition(1). Deiodinase idothyronine typell (Dlo2) are the main factor affect thyroid hormone synthesis, transfer and function(2). In sheep and goats, the expression of *DIO2* is widely distributed in the mediobasal hypothalamus, including the median eminence, arcuate nucleus, infundibular recess, and thyroid(3). Some studies showed that internally timed and spatially regulated changes in *DIO2* and *DIO3* expression may drive the cycling between breeding and nonbreeding states in long-lived seasonal species, and this may be either pars tuberalis-dependent or pars tuberalis independent

at different phases of the circannual cycle(4).

The *DIO2* plays an important role in activation of thyroid hormone by conversion of the prohormone tyroxine (T4) to the active hormone tri iodothyronine (T3)(5). The aim of the present study was to determine the polymorphism distribution and allele frequency of *DIO2* (exon 2) gene and its relationship with the productive traits and prolificacy.

Materials and Methods:

Forty blood samples were collected from local Iraqi Awassi sheep with different ages from (2-5) years old of female ewes located in the farm of Department of animal production/ college of Agriculture engineering science /university of Baghdad during the period from January 2018 to May 2018.All the analysis was done in the laboratories of institute of genetic engineering and biotechnology as well as in the laboratory of college of Agriculture. All the animals enrolled in this study was under a strike nutrition program and veterinary care**.** The oligonucleotide primers sequence were used for amplification of the target DNA as previously described by (2). The sources of the primer that used in this study were BioNeer (korea), the sequence and product size of forward and revers of primers are presented (PCR product Size 350 bp) Figure (1).

F: AAAACAGCGGATGGAAC
R: ACGTTGGCATTATTGAAC

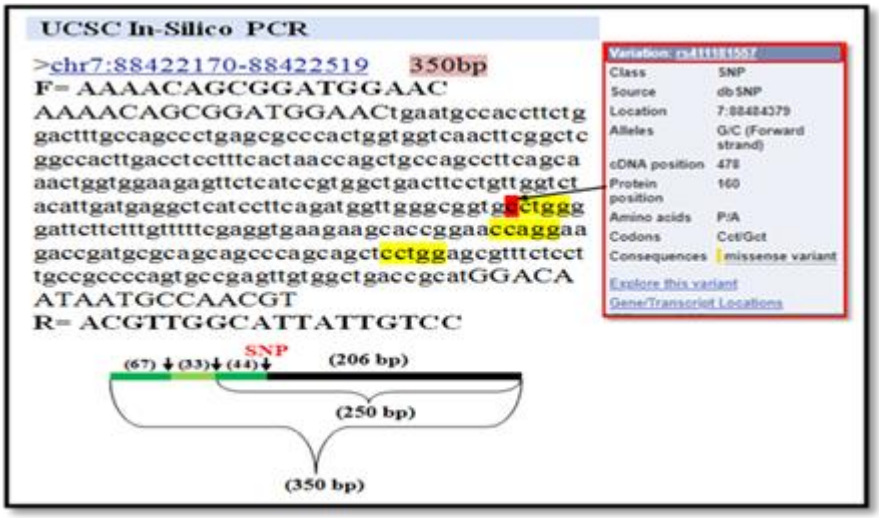

Figure (1): The studied fragment (350 bp) of *DIO2* gene and SNP (rs411181557) studied according to UCSC and NCBI browsers.

Ten ml of blood sample were collected from jugular vein of 40 Iraqi Awaasi sheep by sterile disposable syringes. These sample were divided into two groups:

- **First group:**

Five ml of blood ml put in EDTA tube and stored at deep freezer (- 20 ℃ Until use for DNA extraction (Molecular genetic studies).

- **Second group:**

Include: five ml of blood put in vacationer gel tube without EDTA for serum analysis and then was allowed to clot at 37 ℃ for 30 minutes before being centrifuged. The tubes were

centrifuged at 6000 rpm for 5 minutes; then the serum was collected and kept in freezer (- 20°C) until use for test to detected of the thyroid hormones levels.

Measuring the concentration and purity of DNA:

DNA concentration was measured using by Nanodrop at the DNA center of AI-Nahrain university and concentration was 1.8 where DNA put (1µl) of the DNA extracted was used to detect concentration ng/µl. And purify detected by observing the ratio of optical density (OD) 260/280 nm to detect the contamination of samples with protein. The accepted 260/280 ratio for DNA pure is between 1.8 – 1.9(6).

Agarose Gel Electrophoresis:

The electrophoresis was conducted used according to Sambrook and Russell (6) to detect the presence of DNA bands. This procedure was applied after the extraction of DNA , and to detect the PCR product size as well as the digested product (by restriction enzymes).

Polymerase Chain Reaction (PCR):

PCR was performed using specific primers supplied by Bioneer Company as a lyophilized product . Lyophilized primer has been dissolved in a free nuclease water to give a final concentration of (100 pmol/µl) (as stock solution) to prepare 10 pmol concentration as work primer resuspended 10 µl in 90 µl of nuclease water to reach a final concentration 10 pmol/ µl. To detect the (*DIO2*) gene amplification, the PCR program was adopted in optimal annealing temperature of PCR reaction was find out after several try to be 60°C with a total volume of 25µl.

Detection of PCR Products by Agarose Electrophoresis:

Five µl of amplified products was analyzed by electrophoresis in 2% agarose gel which was stained with 2.5 µg/ml ethidium bromide at 150 volt for 30 mints, in 1XTBE buffer and it was visualized under UV light using ultraviolet transilluminater. A50 bp DNA ladder was used and the gel was photographed by digital camera.

***Bst*N1 restriction enzyme:**

PCR product of 350 bp amplified fragment was digested by *Bst*N1restriction enzyme according to JianNing (2).

Milk collection:

Daily milk production of each ewes located in the station has been measured using ml – graded cylinder twice a month till the end of the production season. As for the total milk production, it has been measured by multiplying daily milk production in the milk seasonality. A milk sample would be taken once a month at morning for three months, after the milk has been weighted, thoroughly mixed and stored in sterile plastic bags (50 ml). The refrigerated bags have been moved to the lab in order to test sampling using a milk content analyzer.

Analysis of milk components:

Collected milk of each ewes was analyzed using special digital milk

analyzer Julie- Z7, which have a digital screen detect proportion of protein, fat, lactase as well as ration of solid non-fatty materials, monthly and for three months.

Statistical Analysis:

Statistical Analysis System-SAS (7) program was used to effect of *DIO2* gene polymorphism with adjusted of fixed factors in study parameters. Chi-square test (χ^2) was used to significant compare between percentage and Duncan (8) multiple range test (ANOVA) was used to significant compares' between means in this study.

Statistical model:

$$Y_{ijklm} = \mu + G_i + A_j + S_k + T_l + e_{ijklm}$$

Y_{ijklm}: Is the m^{th} observation of the genotype i^{th}, age j^{th}, sex k^{th} and l^{th} type of birth. μ: The overall mean of the characteristic. Gi: The effect of polymorphism of *DIO2* gene (CC, CG, GG). A_j: Age of dam effect (2-5 years). S_k: Sex of lambs effect (male, female). T_l: Type of birth effect (single, twin).e_{ijklm} Which is naturally distributed at an average of zero and a variance of $\sigma^2 e$.

Calculator of allele frequency (*DIO2* gene) according of Hardy Weinberg's equilibrium.

Results and Discussion:

The result of recent study demonstrated that concentration ranged from 5-16 ng/µl and purity of the extracted DNA were sufficiently high for PCR Analysis and ranged from 1.8-1.9. Checking of genomic DNA bands on agarose gel electrophoresis was done for detection of integrity and purity as shown in Figure(2).

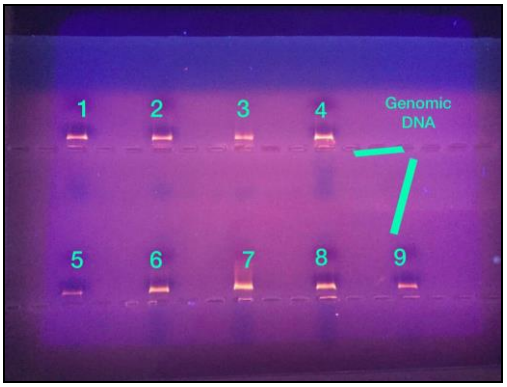

Figure (2): DNA Bands from whole blood Samples Visualized Under UV After Staining with Ethidium Bromide on 1% Agarose gel at 70 volt/cm^2 for 30 Min.

Amplification of *DIO2* gene by polymerase Chain Reaction (PCR):

PCR amplified region of exon 2 on chromosome 14 for *DIO2* gene was improved by using specific primers described by JianNing (2). It has chosen the best temperature for primer annealing (forward and reverse) with DNA template (annealing temperature) with the appropriate time (optimization). After the PCR process was complete, the product electrophoresis on agarose gel (2%). The result show clear bands appeared in specific location compared to ladder with product size (350bp) Figure(3).

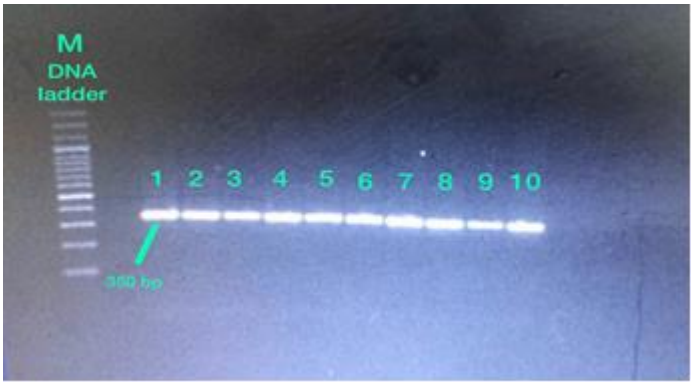

Figure (3): PCR Product of *DIO2* gene, Band Size 350 bp. Electrophoresis on 2% Agarose gel at 150 volt/cm^2 for 60 min, Visualized Under U.V Light After Staining with Ethidium Bromide.
M: 100bp DNA Ladder (100bp). Lone (1-10): amplified DNA from Awassi sheep DNA. That's lodothyronine deiodinases (*DIO2*).

Restriction Fragment Length Polymorphism (RFLP):

RFLP analysis for *DIO2* gene was applied, using digestive (restriction) enzyme *BstNI* on PCR product of *DIO2*, the optimum condition for this enzyme activity at 2 hours incubation, then electrophoreses in agarose gel (3%) for 1 hour. The result showed three genotypes, homozygous GG, band 5 (251bp) ; heterozygous GC , band 6 and 16 (205bp/251bp) and one wild CC, band 10, 12, 13, 14, 15, 18, 19, 20 and 21 (205bp). fragments, as shown in Figure (4).

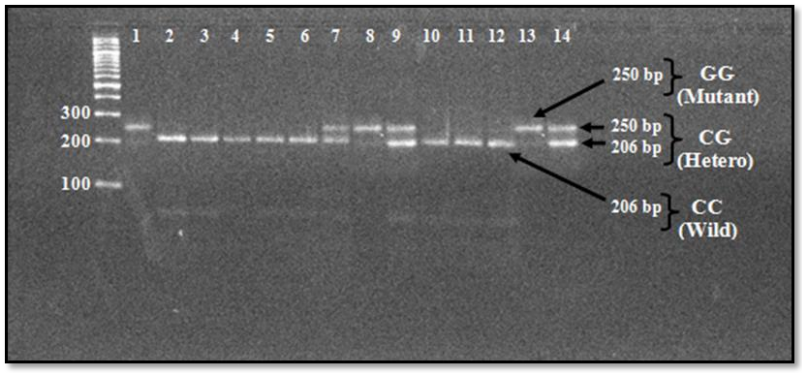

Figure (4): RFLP-PCR electrophoresis for *DIO2* Gene.Shown deferent genotype , electrophoresis on 3% agarose gel at 70 volt for 90 minutes , Visualized Under UV Transilluminator After staining with ethidium bromide.
M: 100bp DNA ladder. Iane: 5 GG(251bp), Iane:6, 16 GC(205bp /251bp), Iane:10, 12, 13, 14, 15, 17, 18, 19, 20, 21 CC(205bp).

Genotype and allele frequencies of *DIO2* gene in sheep:

The distribution of genotype and allele frequency at exon2 of *DIO2* gene presented in Table (1). As shown in this table these is high significance between genotypes of the target gene, as three genetic structures were obtained from the enzymatic product GG, CG, CC, with a ratio of 5.00 %, 12.50%, and 82.50 % respectively .Allele frequencies for C and G were 0. 89, 0.11 respectively.

Table (1): Distribution of *DIO2* gene polymorphism and allele frequency in sample study of Awassi sheep.

Polymorphism	No.	Percentage (%)
CC	33	82.50
CG	5	12.50
GG	2	5.00
Total	40	100%
Chi-Square (χ^2)	---	70.55 **
Allele frequency		
C	0.89	
G	0.11	
**** (P<0.01).**		

The result in this study indicated that the C is the predominant allele and CC was the preponderant genotype in Awassi sheep breed association studies have been in consistent and have produced controversial results(2) who showed that for all pairs of non seasonal breeds there were no consistent significant differences of genotype distribution in each locus of the sheep *DIO2* gene.

Relationship between DIO gene polymorphism with milk production and lactation period:

As in Table (2), the results showed significant differences (P<0.05) in total milk production according to the genotype of *DIO2* gene. The highest mean milk was 82.20 ± 4.21 kg for heterozygous genotype CG, while the mean was 75.02±0.06 kg For ewes with homozygous genotype GG may be attributed to the superiority of the hybrid milk production to the degree of vitality and adaptation. The current study was compatible with the study of Taha (9) of total milk production. While in a study conducted in Iraq (10), the highest average milk production was 127.11±16.53 with genotype AG and the lowest mean was 73.37±19.36 of the genotype GG and also found variation in the seasonal of lactation period was significant (P<0.05) with rates of 112.77 ± 10.49, 130.45 ± 8.17, 105.55 ± 9.77 days for genotypes GG, AG and AA respectively. While the differences were not significant in the length of the lactation period for *DIO2* gene, the rates were 111.15±0.81, 113.60±0.56,110.00±1.02 days for GG, CG, and CC respectively.

Table (2): Relationship between *DIO2* gene polymorphism with total milk production and lactation period in Awassi sheep.

Polymorphism of *DIO2* gene	No. of ewes	Mean ± SE	
		Total milk production (kg)	Lactation period (day)
CC	33	77.33 ± 1.54 ab	111.15 ± 0.81 a
CG	5	82.20 ± 4.21 a	113.60 ± 1.56 a
GG	2	75.02 ± 0.06 b	110.00 ± 1.02 a
Level of sig.	---	*	NS

Means having with the different letters in same column differed significantly.
*** (P<0.05), NS: Non-Significant.**

Relationship between *DIO2* gene polymorphism with major milk contents:

The results of the current study showed that there was a significant effect (P<0.05) on the genetic makeup of the *DIO2* gene in fat ratio fat in milk of (4.93±1.13%) in milk with GG and lowest fat content (2.35±0.04%) that showed in Table (3). This is consistent with the results of milk production. The relationship between milk production and fat ratio is that the GG genotype recorded the lowest milk production and therefore the highest fat , and likewise the GC heterozygous genotype, The lowest fat ratio, which is similar to that of Abbas (10), where the effect was significant (7.81 ± 0.27%) in the ewes milk with the genotype GG (7.05 ± 0.35%) and in the genotype AG, and this is in agree with the results of the study of Alrawi (11) and the highest fat content found by(9,12). Note that fat is one of the most important formulas for milk, which determines the quality of milk, the quality of the product and the type of product it is made of. Useful through the results of this study. At the time, the differences were not significant in the percentage of lactose in milk, the differences were significant in the proportion of protein at 5.45±0.18, 5.93±0.82, 6.80±0.11 for the genetic compositions CC, CG, GG, respectively, and this study exceeded the studies(13,14), in terms of the absence of significant effects in the proportion of protein in sheep. The percentage of non-fatty solids significantly affected the genotype of *DIO2* gene and the maximum 11.61±0.27 for GG and 10.79±0.24 for CC.

Table (3): Relationship between *DIO2* gene polymorphism with major milk contents in Awassi sheep.

Polymorphism of *DIO2* gene	No. of ewes (sample)	Mean ± SE			
		Fat in milk (%)	Lactose in milk (%)	Protein in milk (%)	Sold non-fat (%)
CC	33 (99)	3.91 ± 0.44 ab	4.44 ± 0.03 a	5.45 ± 0.18 b	10.79 ± 0.24 b
CG	5 (15)	2.35 ± 0.04 b	4.47 ± 0.05 a	5.93 ± 0.28 b	11.16 ± 0.38 ab
GG	2 (6)	4.93 ± 1.13 a	4.41 ± 0.08 a	6.80 ± 0.11 a	11.61 ± 0.27 a
Level of sig.	---	*	NS	*	*

Means having with the different letters in same column differed significantly. * (P<0.05), NS.

Relationship between *DIO2* gene polymorphism with litter size:

Table (4) explain the relevance of the genotypes with the litter size with a significant effect (P <0.05) by different genotypes of the *DIO2* gene with the highest twining rate (1.20±0.05) for CG, followed by ewes with CC (1.15±0.05), And ewes with GG had a minimum litter size (1.00±0.00). Another study conducted by (2) with the highest litter size (2.24±0.23) for the TT genotype, followed by GT (2.39±0.20) and the lowest twining rate (2.32±0.09) for the GG genotype. who showed no significant association between polymorphism and litter size of small tail han sheep in chain. As *DIO2* may be involved in photo periodic control of seasonal breeding based on the melatonin signal messenger that in temperate Areas, the reproductive activities of mammals are controlled by photoperiod rhythm (15) found that reciprocal changes in *DIO2* and DIO3

expression act as gene switches of the photoperiodic molecular cascade to trigger introduction of luteinizing hormone secretion, In Japanese quail as along day –breeds his is different for short day-breeds (16).

Table (4): Relationship between *DIO2* gene polymorphism with litter size.

Polymorphism of *DIO2* gene	No. of ewes (40 ewes)	No of lambs (46 lambs)	Mean ± SE
CC	33	38	1.15 ± 0.06 a
CG	5	6	1.20 ± 0.05 a
GG	2	2	1.00 ± 0.00 b
Level of sig.	---	---	*

Means having with the different letters in same column differed significantly. * (P<0.05).

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