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NOVEL ASSOCIATION BETWEEN GnRHR GENE AND GROWTH TRAITS IN AWASSI AND KARAKUL SHEEP

Thamer R. Al-Jubouri[1], *Amad F. Hassan*[2], *Mohammed Baqur S. Al-Shuhaib**[1]

Address(es): *Dr Mohammed Baqur S. Al-Shuhaib,*
[1]Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim, Babil 51001, Iraq.
[2]Department of Animal Production, College of Agriculture, University of Basrah, Basrah, 61001, Iraq.

*Corresponding author: mohammed79@agre.uoqasim.edu.iq

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ABSTRACT

This study was conducted to investigate the association of the Gonadotropin-Releasing Hormone Receptor (*GnRHR*) gene with growth traits in Awassi (n= 123) and Karakul (n= 78) sheep. Starting from the birth-day to 12 months of age, several growth traits were investigated, namely body weight and length, wither and rump height, chest, and abdominal circumferences. Four fragments were designed to amplify three exons of *GnRHR* gene, P1 and P2 for exon 1, P3 for exon 2, and P4 for exon 3. The polymorphisms in these amplicons were analyzed by single-strand conformation polymorphism (SSCP) method. Genotypic and allele frequency of *GnRHR* gene were computed after sequence alignment. The results detected two different SSCP banding patterns GG and GA for both P2 and P3 amplicons, and two novel mutations were observed in GA genotype of P2 and P3 amplicons respectively, p.N87= and p.L242F. The identified p.N87= exhibited no relationship with all measured growth traits, while p.L242F showed significant association with the body weight, length, and abdominal circumference in month-6 and month-9. This association was increasingly observed in month-12 with all growth traits, in which individuals with GA genotype exhibited higher growth traits measurements than individuals with GG genotype ($P < 0.01$). In conclusion, the detected polymorphism of *GnRHR* gene exon-2 may be useful in marker-assisted selection for growth traits in Awassi and Karakul breeds. This study is the first one to describe the effect of *GnRHR* polymorphisms on growth traits, which confirm the potential usefulness of this gene in marker-assisted selection in sheep.

Keywords: Awassi; *GnRHR*; growth; Karakul; polymorphism; sheep; traits

INTRODUCTION

Sheep farming is of major economic importance in the middle east. The sheep industry has become the primary source of income for breeders because it requires minimal resources. Improvement of growth traits of sheep is the main concern of many researchers nowadays (**Lalit *et al.*, 2016**). Growth trait is the most important economic trait in domestic animals and is controlled by multiple genes, loci, and interactions (**Cheng *et al.*, 2011**). Growth of the lambs is a reflection of the economic viability and adaptability of sheep to the environment and can be used as a criterion for the selection among breeds and the individual within breeds (**Singh *et al.*, 2006; Al-Thuwaini *et al.*, 2020a**). One of these genetic factors that have a possible association with growth traits is the Gonadotropin-Releasing Hormone Receptor (*GnRHR*) gene. This gene is located in chromosome 6 in the ovine genome and consists of three exons separated by two introns (GenBank acc. no. NC_019463.2). The GnRHR protein, the product of the *GnRHR* gene, is a member of the G protein-coupled receptor family and is made of 328 amino acids that are secreted from the pituitary gonadotrophic gland (**Millar et *al.*, 2004**). It has a fundamental role in regulating the synthesis and release of the luteinizing hormone (LH) and follicle-stimulating hormone (FSH), the key regulators of steroidogenesis and gametogenesis (**Finch *et al.*, 2008**). It has been known that GnRHR mediates the action of GnRH to stimulate the production of gametes and gonadal hormones (**Sealfon *et al.*, 1997**). In addition to its role in reproduction, it has been well-known that GnRHR is involved in different developmental and metabolic processes (**Busby and Sherwood, 2017**). Furthermore, there are accumulated pieces of evidence have increasingly been concerned with the importance of the GnRHR protein to act as a key element in the normal development of puberty (**Zhang *et al.*, 2018**). Most mutations in *GnRHR* that either activate or inactivate their functions were reported to be responsible for several reproductive traits in domestic animals, such as cattle (**Kerekoppa *et al.*, 2015**), buffalos (**Sosa *et al.*, 2016**), and goats (**Bemji *et al.*, 2018**). Thus, the investigation of single nucleotide polymorphisms (SNP) within this gene may be important to understand the possible causative SNP(s) behind a particular phenotypic trait. It is well-established that synonymous, or silent mutations may cause variable alterations in metabolic activities (**Im *et al.*, 2018**). Though there is no precise mechanism to clarify how silent mutations induce such functional impact (**Zheng *et al.*, 2019**), it could change the substrate specificity of proteins (**Kirchner *et al.*, 2017**). However, silent mutations - do not alter amino acid sequences, while nonsynonymous single nucleotide polymorphism, or nsSNP, possess a straightforward effect on the biological function of a protein by modifying its amino acid sequences. In this way, such variants can modify protein structure and enzyme activity, and alter protein stability, or interact with protein binding capacity. Thus, nsSNPs have been involved in the final manifestation of encoded proteins and have been associated with various physiological and phenotypic effects (**Yakubu *et al.*, 2017**). However, there are several single nucleotide polymorphisms (SNPs) reported for *GnRHR* in the literature (**Flanagan and Manilall, 2017**), but only 6 SNPs change the amino acid sequence in the gene (D8N, Q65P, I169V, R240S, Q244E, Q260R) (https://asia.ensembl.org). These detected nsSNPs may have a significant positive or negative impact on growth traits performance in several breeds of sheep. However, the relationship of these coding regions of *GnRHR* with the growth traits in sheep is still not widely known. Therefore, it is necessary to analyze the possible effect of *GnRHR* genetic polymorphisms on the altered amino acid sequences, as well as to incorporate knowledge of *GnRHR* polymorphisms in the growth traits. Despite the reported remarkable association between the *GnRHR* sequences and several production traits in sheep (**Sun *et al.*, 2008**), the associations between *GnRHR* polymorphism and growth traits in sheep have not been reported yet. Keeping in view of this aspect, the present study was envisaged to detect the possible SNPs in the coding regions of the *GnRHR* gene using polymerase chain reaction–single-strand conformation polymorphism (PCR-SSCP) and DNA sequencing methods, and to investigate the association of *GnRHR* genes mutations with growth traits in two types of sheep breeds.

MATERIALS AND METHODS

The Experimental protocol on sheep comply with international guidelines and was approved by the international recommendations for the care and use of animals. Both maintenance and feeding were similar for all animals and remained in accordance with proper animal welfare guidelines for the care and use of agricultural animals (**Vaughn, 2012**).

Animals

This study was conducted on two breeds of lambs (123 Awassi and 78 Karakul). Both breeds were born and reared under the same conditions at Barakat Abu al Fadhl Al-Abbas Station (BAFAS) for raising sheep (Al-Khafeel co., Karbala, Iraq). All lambs were ear-tagged within 12 h of birth and allowed to stay with their dams until weaning for approximately three months of age. All animal data were collected by the same staff in the specified breeding station, which were recorded in every three months' intervals, beginning from the birthday until one year of age (birthday, 3 months, 6 months, 9 months, and 12 months). These records included body weight and length, wither and rump height, chest and abdominal circumferences, and average daily gain.

Genomic DNA extraction

At weaning, jugular blood samples of 201 lambs (2 ml each) were collected in vacutainer tubes containing EDTA as anticoagulant and kept in ice. Genomic DNA was isolated from peripheral blood cells using a rapid salting-out method as per the standard protocol described by **Al-Shuhaib** (**2017**). Both the quality and quantity of the extracted DNA samples were assessed by Nanodrop spectrophotometer (Biodrop, µLITE, UK), while the integrity was also assessed by running on 0.8% agarose gel.

Primer design and polymerase chain reaction amplification

According to the reference sequence provided by GenBank accession number NC_019463.2, all the coding regions of *GnRHR* gene were amplified using four pairs of specific primers. These primers were designed by NCBI-Primer BLAST server for amplification of exon 1 (P1 and P2), exon 2 (P3), and exon 3 (P4), respectively (Figure 1, A) (**Ye *et al.*, 2012**). The sequences of the designed primers were described in (Table 1).

Table 1 The designed primers of the *GnRHR* gene by NCBI primer BLAST tool based on the acc. no. NC_019463.2

Exon No.	Primers' code	Sequence (5′-3′)	Length (bp)	Start	Stop	Annealing temp.
Exon 1 (first half)	*P1* - F	TGGTTTACCTGTGGTCCAGC	246 bp	83314086	83314105	60.1˚C
	P1 - R	GCCACTGGATGGAATGTGGA		83314656	83314636	
Exon 1 (second half)	*P2* - F	GGGGGCGTACATGGAGAAAA	388 bp	83314232	83314251	60.9˚C
	P2 - R	AAGGATGGCAAACGGTGACT		83314600	83314619	
Exon 2	*P3* - F	GGCACAGCAAGTGCCATTC	435 bp	83302044	83302062	59.4˚C
	P3 - R	GCAGTTGCTTTGGGCAACAA		83302478	83302459	
Exon 3	*P4* - F	GCTTTGCGTTTCTCATTCCCA	432 bp	83298246	83298266	60.9˚C
	P4 - R	TGCCTCCATTATATTCACCAGGTT		83298677	83298654	

Genomic DNA samples of 201 lambs were adjusted to a concentration of 100 ng/µL and exactly 0.7 µL of each DNA sample was used as a template for PCR. The amplification procedure for *GnRHR* gene of sheep was empirically standardized by a gradient PCR thermocycler to yield consistent and specific amplification. The amplification reaction conditions were carried out using Bioneer PCR premix (Daejeon, South Korea). PCR thermocycling experiments were conducted using 30 cycles at 94°C for 5 min, followed by 94°C for 30 s, 59.4°C – 61.1°C for 30 s, 72°C for 30 s, followed by 72°C for 5 min. PCR amplicons specificity with the targeted loci were confirmed by running on 1.5% agarose gel in parallel with DNA marker before being analyzed by SSCP (Figure 1, B).

Single-strand conformation polymorphism analysis

The coding region variation of *GnRHR* was performed by a rapid SSCP protocol described by **Al-Shuhaib *et al.*** (**2019**) with minor modifications. One µl PCR amplicons and an equal volume of SSCP denaturing-loading buffer (95% formamide, 20mM EDTA pH 8, 0.05% xylene cyanol, and 0.05% bromophenol blue) were denatured at 97°C for 7 min and immediately kept in ice for at least 10 min. The denatured PCR products were run in a non-denaturing 8% polyacrylamide gel for 4 h at 200 V/100mA in 20x20x0.1 (length, width, gel thickness) vertical gel module. After electrophoresis, SSCP gels are fixed and stained by silver nitrate according to the method described by **Byun *et al.*** (**2009**). Each detected SSCP pattern was recorded and selected for downstream sequencing reactions.

DNA sequence analysis

PCR products showing different banding patterns on SSCP gel were selected for sequencing. The selected PCR amplicons were exposed to custom sequencing in both termini using DNA sequencing service provided by Macrogen (Geumchen, Seoul, South Korea). Sequence alignments were conducted by BioEdit ver, 7.1. (DNASTAR, Madison) to identify SNPs, while translations to proteins and comparisons of amino acid sequences were carried out by Expasy translate and UniProtKB suits, respectively (**Gasteiger *et al.*, 2003**; **UniProt Consortium, 2008**). Sequence variation chromatograms were annotated by SnapGene Viewer Ver. 4.0.4, (GSL Biotech, http://www.snapgene.com). The reference amino acid sequences of the ovine GnRHR protein were retrieved from Uniprot server (https://www.uniprot.org/uniprot/P32237) and the 3-dimensional structure of GnRHR protein was generated using RaptorX software (**Källberg *et al.*, 2012**). The detected amino acid substitution was highlighted in the 3-dimensional structure of GnRHR using PyMol software ver. 7.0.1 (The PyMOL Molecular Graphics System, Schrödinger, LLC.).

Genetic diversity analysis

The genotypic frequency was determined by the formula $p = n / N$, where p is the frequency of genotype determination, n is the number of individuals with a particular genotype, and N is the number of all individuals (**Gorlov *et al.*, 2017**). The frequency of different alleles of the *GnRHR* gene, heterozygosity observed (*Ho*) and heterozygosity expected (*He*) values, and Chi-square test were calculated by Popgene32 Software ver. 1.31 (**Yeh *et al.*, 1999**). The expected genotype frequencies were calculated by the Hardy–Weinberg law. The polymorphism information content (*PIC*) was computed using the following formula (**Bolstein *et al.*, 1980**).

$$PIC = 1 - \sum_{i=1}^{m} p_i^2 - \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} 2 p_i^2 p_j^2 ;$$

where *pi* and *pj* are the frequencies of the *ith* and *jth* allele, respectively, and *m* is the number of alleles.

Statistical analysis

The associations between the detected genotypes and data of different growth traits obtained from the birthday to 12 months were statistically analyzed in both breeds using the following general linear mixed model which was employed by SPSS, ver. 23.0 (IBM, NY, USA); $Y_{ij} = \mu + G_i + A_j + e_{ij}$, where Y_{ij} is the phenotypic value of the animal, μ is the overall mean effects of the population, G_i is the effect of the genotype, A_j is the age effect, and e_{ij} is the stochastic error. The effects associated with the breed, sex, and type of birth of lambs were not matched in the linear model, as the preliminary statistical analyses indicated no significant influence of these factors on the variability of traits in the analyzed breeds. Statistical significance of differences between genotypes was evaluated with the T-test.

RESULTS

Polymerase chain reaction–single-strand conformation polymorphism analysis

PCR-SSCP technique was efficiently employed to identify unknown genetic variants of four amplicons (P1 – P4) of the *GnRHR* gene in two types of sheep breeds **Hashim and Al-Shuhaib, 2019**). With regards to both P1 and P4 amplicons, no detectable variations were observed and only homogeneous SSCP-banding patterns were observed. Considering both P2 and P3 amplicons, results revealed two SSCP banding patterns for PCR amplicons in non-denaturing 8% polyacrylamide gel, which was represented by two and three bands for both P2 and P3 amplicons (Figure 1, C).

Sequencing analysis

The PCR amplicons corresponding to each of the different PCR-SSCP patterns were selected and sequenced using the forward and reverse primer to detect variations. The results obtained by DNA sequencing showed a transition of G → A at position 96 of the P2 amplicons of the *GnRHR* gene with a silent effect (both encode Asparagine at 87th amino acid residue of GnRHR) (Figure 1, D). The same transition of G → A was also detected in P3 amplicons at position 104

with a missense effect (Leu → Phe at 242th, p.L242P, amino acid residue of GnRHR). According to this detected nucleic acid substitution, both SNPs were assigned GG for individuals with two SSCP bands and GA for individuals with three SSCP bands, respectively. In Figure 1, E, the pattern of this amino acid substitution was highlighted side by side in the 3-dimensional structure of both wild-type and mutant GnRHR protein, respectively. In the 242 position of the mature GnRHR, an amino acid substitution of Leu (aliphatic residue) to Phe (aromatic residue) occurred. Both wild and mutant amino acid residues differ in biochemical properties as Phe can exhibit both polar and non-polar characters. The novelty of the observed variations was confirmed by viewing the corresponding *GnRHR* sequences using the NCBI database as well as ensemble genome browser 99 (https://asia.ensembl.org). The representative sequences of GG and GA genotypes were deposited into the NCBI GenBank database under the accession numbers MN566456 and MN566457 for P2 amplicons and MN566458 and MN566459 for P3 amplicons, respectively.

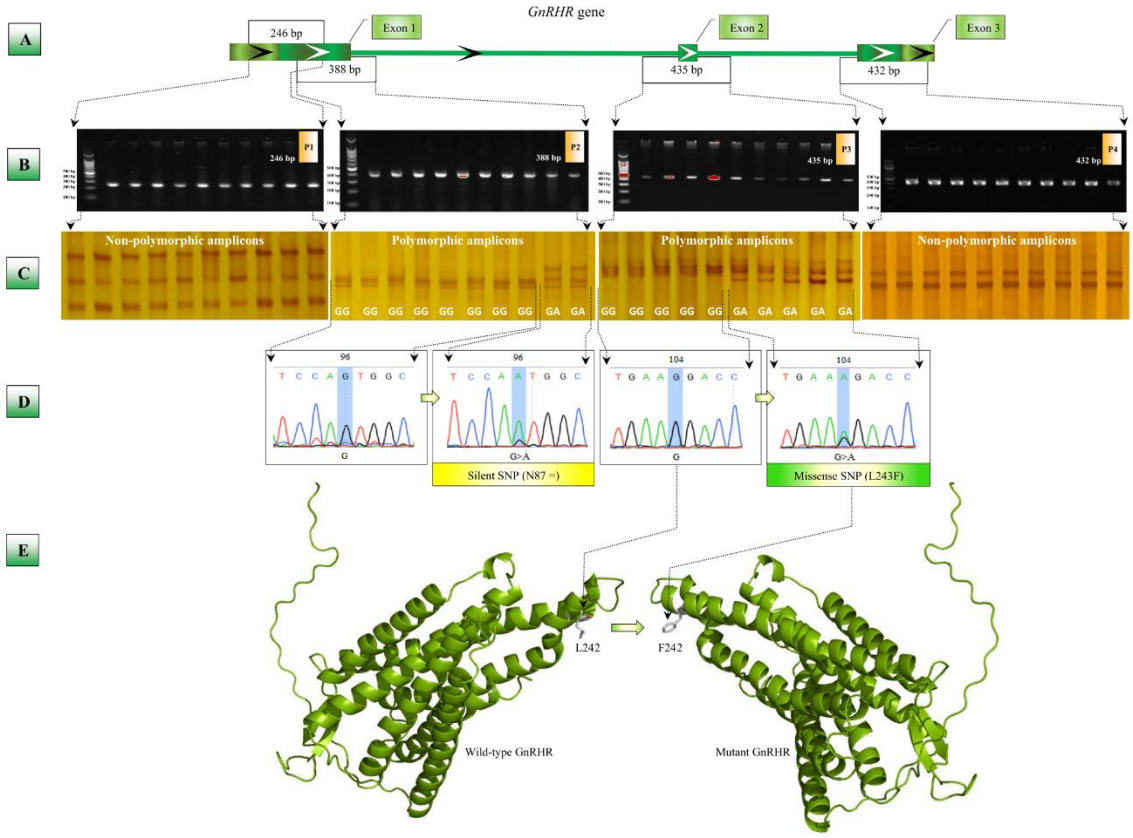

Figure 1 PCR-SSCP sequencing overflow of the *GnRHR* genetic polymorphisms in Awassi and Karakul breeds. A; PCR primers design for P1 – P4 amplicons to cover exon 1 - exon 3 regions within the targeted ovine *GnRHR* gene. **B;** the confirmation of PCR specificity of the amplified loci in parallel with DNA marker. **C;** The SSCP banding patterns of P1 – P4 amplicons. **D;** Electrocardiograms of the observed nucleic acid substitutions in both P2 (G96A) and P3 (G104A) amplicons. **E;** The positioning of P3 (G104A) missense p.L242F mutation in the 3-dimensional structure of GnRHR

Genotype distribution, genotype and allele frequencies

Concerning genotype variations in P2 amplicons, it was found that out of 123 Awassi lambs, 106 samples had GG genotype and 17 samples had GA genotype, while out of 78 Karakul lambs, 67 had GG genotypes and 11 had GA genotypes (in the exon 1 of *GnRHR* gene). Whereas the genotype variation of P3 amplicons (in the exon 2 of *GnRHR* gene) took a different distribution, in which 60 samples had GG genotype and 63 samples had GA genotype in Awassi lambs, while 63 had GG genotypes and 15 had GA genotypes in Karakul lambs (Table 2).

Table 2 Genotype and allele frequencies and genetic diversity parameters for the *GnRHR* gene in Awassi and Karakul breeds.

Breed	Amplicon	Observed genotypes		Genotype frequencies		Allele frequencies		*Ho*	*He*	*Ne*	*PIC*	$\chi 2$
		GG	GA	GG	GA	G	A					
Awassi	P2	106	17	0.86	0.14	0.9309	0.0691	0.1382	0.1292	0.1287	12.04	0.63556
Karakul		67	11	0.86	0.14	0.9295	0.0705	0.1410	0.8681	0.1311	12.25	0.40565
Awassi	P3	60	63	0.49	0.51	0.7439	0.2561	0.5122	0.3826	0.3810	30.8	14.3076
Karakul		63	15	0.80	0.20	0.9038	0.0962	0.1923	0.1749	0.1738	15.88	0.81914

Abbreviations: $\chi 2$ – chi-square, *Ho* –heterozygosity observed, *He* –heterozygosity expected, *Ne*- effective allele number. All Chi-square tests have one degree of freedom and within the significance level $P < 0.05$.

The results of Chi-square tests were shown that both P2 and P3 amplicons were not fitted with the Hardy–Weinberg equilibrium ($P < 0.05$) as for years these breeds were under meat production selection. Concerning P2 amplicons, the values of the *Ho*, *He*, *Ne*, and *PIC* for Karakul breed were higher than that found in Awassi breed, signifying a higher level of genetic variations in Karakul breed in this locus. In contrary to P2, the values of the *Ho*, *He*, *Ne*, and *PIC* for Awassi breed were higher than Karakul breed, which implied a higher level of genetic variation in Awassi breed in P3 locus. However, *PIC* values at P2 locus showed low genetic polymorphisms in both breeds (12.04 in Awassi and 12.25 in Karakul). Concerning P3 locus, Karakul breed showed moderate levels of genetic variations (*PIC* = 15.88), while Awassi breed exhibited high levels of genetic polymorphisms (*PIC* = 30.8). This observation indicated higher genetic polymorphisms in the analyzed P3 locus than the P2 locus.

Association analysis

A noticeable association between the two observed genotypes of P3 amplicons and the majority of growth traits was identified in this study. This association was initiated by the presence of highly significant differences ($P < 0.01$) in body weight and length, and chest circumferences of lambs with GA genotypes than lambs with GG genotype in both month-6 and month-9. The superiority of lambs with GA genotype over lambs with GG genotype was increasingly observed in month-12 to include all measured growth traits (Table 3). These significant differences indicated that individuals with GA genotype had higher growth indices than individuals with GG genotype. Meanwhile, results showed no significant association ($P < 0.05$) of P2 amplicons with all growth traits recorded every three months' intervals.

Table 3 Genotypes of *GnRHR* gene associated with growth traits in Awassi and Karakul sheep.

		P2 amplicons		P3 amplicons	
Age	**Traits**	**GG (173)**	**GA (28)**	**GG (123)**	**GA (78)**
Born	Body weight	4.368±0.049	4.419±0.132	4.417±0.074	4.350±0.058
	Body length	30.50±0.157	30.14±0.443	29.99±0.197	30.72±0.202
	Wither height	40.09±0.131	39.96±0.441	40.08±0.197	40.07±0.168
	Rump height	40.43±0.137	40.32±0.422	40.43±0.196	40.40±0.174
	Chest circumference	41.34±0.175	41.86±0.516	41.75±0.266	41.21±0.213
	Abdominal circumference	42.90±0.190	43.79±0.475	43.25±0.268	42.88±0.234
Month - 3	Body weight	21.61±0.303	21.58±0.754	21.09±0.422	21.91±0.379
	Body length	56.72±0.316	57.69±0.796	56.18±0.452	57.25±0.382
	Wither height	61.05±0.268	61.77±0.701	60.84±0.418	61.33±0.313
	Rump height	61.76±0.254	62.27±0.698	61.53±0.390	62.01±0.303
	Chest circumference	76.48±0.416	76.00±0.992	75.58±0.627	76.92±0.483
	Abdominal circumference	84.23±0.439	83.81±0.969	83.35±0.585	84.67±0.534
	Average day gain (g)	194.810±5.30	191.058±9.370	192.047±7.709	193.821±6.522
Month - 6	Body weight	29.70±0.357	28.426±0. 817	26.575±0.445 ^{B}	34.192±0.737 ^{A}
	Body length	68.061±0.422	67.271±0.964	65.622±0.525 ^{B}	70.523±0.869 ^{A}
	Wither height	68.738±0.306	67.401±0. 699	67.314±0.381	69.300±0.631
	Rump height	69.261±0.309	67.729±0.706	67.590±0.384 ^{b}	69.956±0.637 ^{a}
	Chest circumference	86.946±0.870	84.195±1.989	82.724±1.083 ^{B}	89.824±1.794 ^{A}
	Abdominal circumference	97.395±0.909	95.963±2.078	93.287±1.132 ^{B}	101.441±1.874 ^{A}
	Average day gain (g)	94.980±4.942	90.459±8.735	91.083±7.161	94.356±6.089
Month - 9	Body weight	35.584±0.372	33.313±0.850	31.205±0.463 ^{B}	39.153±0.766^{A}
	Body length	71.560±0.346	69.987±0.792	69.323±0.431 ^{B}	72.969±0.714^{A}
	Wither height	71.264±0.286	70.601±0.653	69.688±0.355	71.536±0.589
	Rump height	71.689±0.290	71.490±0.662	69.932±0.360	71.833±0.597
	Chest circumference	96.912±0.938	91.517±2.144	92.039±1.167	98.014±1.933
	Abdominal circumference	107.275 ±1.05	106.476±2.415	102.022±1.31 ^{B}	113.480±2.177 ^{A}
	Average day gain (g)	52.050±2.316	54.302±5.295	51.438±2.883	55.119±4.775
Month - 12	Body weight	39.807±0.389	37.456±0.888	34.723±0.484 ^{B}	44.236 ±0.801 ^{A}
	Body length	73.676±0.308	72.418 ±0.704	71.664±0.383 ^{B}	75.100±0.635 ^{A}
	Wither height	73.043±0.267	71.685±0.610	71.406±0.332 ^{B}	73.868±0.551 ^{A}
	Rump height	73.286±0.273	71.896±0.624	71.700±0.340 ^{B}	74.010±0.563 ^{A}
	Chest circumference	105.18±0.868	100.918±1.985	99.934±1.081 ^{B}	107.911±1.790 ^{A}
	Abdominal circumference	116.945±0.99	114.372 ±2.269	111.220±1.23 ^{B}	122.005±2.046 ^{A}
	Average day gain (g)	46.925±1.900	46.034±4.343	39.092±2.364 ^{B}	56.478±3.916 ^{A}

Data in the table; means ± standard error. Values with different superscript letters within the same line differ significantly at $P < 0.01$ (A, B) and $P < 0.05$ (a, b).

DISCUSSION

The present study describes the association between the *GnRHR* genetic variations and growth traits measurements in two breeds of lambs, Awassi, and Karakul. Awassi breed is the main fat-tailed breed in the eastern region of the Mediterranean. This breed has multiple beneficial characteristics, including its higher resistance to environmental fluctuations and diseases, and tolerance of extreme temperatures than other breeds (**Galal *et al.* 2008**). However, Karakul breed has higher reproductive performance than Awassi breed (**Aljubouri and Al-Shuhaib, 2020**). Therefore, the investigation of *GnRHR* genetic polymorphism between both breeds may be interesting to assess the role of this genetic locus in such differences.

Using four sets of amplicons, P1 – P4, polymorphisms of *GnRHR* gene coding regions were detected by PCR-SSCP and DNA sequencing, and the association with growth traits of both Awassi and Karakul breeds were analyzed. Concerning both P2 and P3 amplicons, our results showed the presence of only two genotypes in the analyzed animals (GG and GA), while no animal with AA genotype was identified. The reason for the absence of this genotype may be attributed to several factors, such as the number of animals studied, the small number of animals with a specific genotype, breed composition differences, or population differences (**Moussavi *et al.*, 2006**). No heterogeneous SSCP banding patterns of both P1 and P4 amplicons were found. This monomorphous pattern of these amplicons that designed to screen the first portion of exon 1, and the entire exon 3 suggested no relationship between these coding loci and the measured growth indices. In contrary to P1 and P4 amplicons, heterogenic SSCP banding patterns were detected in both P2 and P3 amplicons, with a silent p.N87= and a missense p.L242F SNPs in P3 and P4 amplicons, respectively. However, the detected genotypes distributions of P2 amplicons implied no significant relationship between p.N87= and all measured growth traits in all the analyzed time intervals. In contrary to p.N87= SNP, p.L242F SNP was found to be associated with some growth traits before sexual maturity and all traits after sexual maturity. Therefore, individuals with GA genotype (having p.L242F SNP) are more favoured than individuals with GG genotype (wild-type) due to their significant superiority in terms of measured growth traits. This observation entails increasing significant associations of the *GnRHR* genotypes with growth traits which were detected in post-sexual maturity. These findings suggested that p.L242F SNP may influence numerous patterns of GnRHR protein metabolism involved in the development of puberty. This finding implies that the currently observed genetic polymorphisms of the *GnRHR* exon 2 are found to take a major role in the development of growth traits. This essential role is may be due to the specific control of GnRHR on the sort of sexual maturity of sheep (**Malcolm *et al.*, 2006**). This finding might be in agreement with those who also reported the contribution of genetic variation of GnRHR in the regulation of pubertal timing in human and mouse populations (**Juan *et al.*, 2012**). However, the number of GnRHRs on the plasma membrane varies with age, sex, and physiological status (**Janjic *et al.*, 2017**). Hence, the detected p.L242F amino acid substitutions within *GnRHR* may be involved in the significant alterations found in growth traits, which may lie behind the positive attitude of GA genotype. However, we also supported this suggestion by utilizing a set of state-of-the-art *in silico* tools to predict the effect this SNP on GnRHR structure and function. The predictions with SIFT (**Pauline and Steven, 2003**), PROVEAN (**Choi *et al.*, 2012**), PhD SNP (**Capriotti et *al.*, 2006**), and ConSurf servers showed non-deleterious consequences of p.L242F on the altered GnRHR protein. Because both leucine and phenylalanine exerted non-similar R- groups, the observed amino acid substitution of p.L242F may exhibit a noticeable effect on protein structure, function, and stability (**Al-Shuhaib, 2019**). Because of this clear biochemical conversion, it can be inferred that the p.L242F SNP may directly influence the growth traits of the studied sheep populations (**Al-Thuwaini *et al.*, 2020b**).

Though has not been studied yet in sheep, *GnRHR* gene polymorphism has been proven efficiency in association with reproductive performance in several breeds of goats, such as Chinese goats (**An *et al.*, 2009**), Boer goats (**Yang *et al.*, 2011**), Loashan dairy goats (**Liu *et al.*, 2014**), and Shaanan goats (**Li *et al.*, 2011**). Furthermore, high genetic variation of *GnRHR* has also been reported in both Holstein Friesian cattle and Malnad Gidda buffalos (**Kerekoppa *et al.*, 2015**), and

in Egyptian buffalos (**Sosa *et al.*, 2016**). However, no previous study was conducted to describe the possible association of the GnRHR with growth traits of sheep to compare our results with. Therefore, it can be stated that this study describes a novel association between p.L242F SNP and growth traits in the advent of puberty, which paves the way for further understanding of *GnRHR* polymorphism in other breeds of sheep as well as other livestock populations.

CONCLUSION

The genotyping experiments were conducted for all the coding regions of the *GnRHR* gene, P1 – P4 amplicons. Both P2 and P3 amplicons were found to be polymorphic with GG and GA genotypes. P2 amplicons did not exert any significant association with growth traits, while P3 amplicons exhibited a clear association with all growth traits in the advent of sexual maturity. Animals with GA genotype have better growth traits indices than animals with GG genotype. In GA genotype, the novel p.L242F SNP has been predicted to be linked with such observed differences in growth traits. Therefore, the variation of *GnRHR* gene exon 2 is strongly suggested as a promising candidate for the assessment of growth traits in sheep.

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