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Association of the ANXA9 gene with milk production and its components in Awassi sheep

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Abstract

The study was conducted in the sheep farm of the Al-Fayhaa station in the Jableh sub-district / Al-Musaib project (55 km south of Baghdad), as well as the Biotechnology Laboratory in the College of Agricultural Engineering Sciences / University of Baghdad for the period from 5/1/2022 to 30/10/2022. With the aim of detection the ANXA9 gene polymorphism and its relationship to Daily milk yield (DMY), Lactation period and milk composition, as well as the Polymorphism distribution and allele frequency in 52 Awassi sheep and its lambs, three polymorphism appeared in this variant (T>G SNP) which are TT, TG and GG, and their percentage were 51.92, 40.38 and 7.69%, and the differences between them were highly significant (P≤0.01), with a frequency of 0.72 and 0.28 for the T and G alleles, respectively. the daily milk production rate of Awassi ewes was affected significantly (P≤0.05) by the ANXA9 gene polymorphism, the first variation (T>G SNP), for ewes with the TG polymorphism (1128.65 g), while the components of milk were not affected significantly except for the percentage of Fat, which was maximum in the milk of ewes for GG and TT genotype (5.67 and 5.64%).We can conclude by studying the ANXA9 gene polymorphism that they can be adopted in developing strategies for genetic improvement of sheep, and the application of the study to a larger sample and to several sites and extracting the interaction between two SNPs would give more accurate results and determine the best method for managing and improving sheep flocks.

Keywords: Awassi sheep, ANXA9 gene, Daily milk yield, Lactation period, milk composition.

Introduction

The scarcity of pedigree and management becomes an important, which is required for genetic evaluation and reliable selection decisions, is a major barrier to improving the genetic traits of small farmers' flocks in developing livestock systems [1, 2]. Genetic selection has evolved into an important approach in genetic improvement [3, 4, 5, 6, 7]. The ANXA9 gene encodes the protein Annexin 9 (ANXA9). This protein binds to phospholipids as well as Ca+2. This protein is also involved in cytoplasmic membrane transfer, which is vital in the mammary gland [8, 9]. Annexin 9 protein (ANXA9) has several

roles in the cell, including signal processing, endocytosis, calcium channel creation, and cell cycle control of the inflammatory response. The ANXA9 gene has been shown to be expressed in a variety of tissues, notably those with fast acid metabolism. Adipose tissue, for example, breast tissue during breastfeeding [10]. Annexins have been suggested as membrane-membrane or membrane-cytoskeleton linkers, and have been linked to Ca^{2+} regulated exocytosis, and plasma membrane domains, Furthermore, annexins are assumed to function as Ca^{2+} channels, but how calcium solubility is accomplished remains unknown [11]. The ANXA9 gene in sheep was identified on chromosome 1, within microsatellite markers INRA006 and AE57 [12]. In this area the QTL for protein content in milk and milk production were also identified [13, 14]. The aim of this study to determination of the ANXA9 gene polymorphism and its relationship with daily milk yield, lactation period and milk composition in Awassi sheep.

Materials and Methods

In the current study, 52 female sheep (ewes) and their lambs are used. During the period 5-1-2022 to 25-6-2022, samples were collected in a sheep farm / Al-Fayha station in Jableh sub-district / Al-Musaib project (55 km south of Baghdad city). The goal of this study was to separate the genetic information and determine the genotype of the ANXA9 gene and its relationship to daily milk yield, lactation period, and milk composition in Awassi sheep, as well as to study the percentages of the distribution of their constructions in the herd and the intensity of alleles obtained. Five milliliters of blood were drawn from the jugular vein. [15, 16, 17] for each sheep in a collection tube supplemented with a Jordanian company's (AFCO) K2 EDTA anticoagulant, and transmitted in a cool box to the lab (the Biotechnology Laboratory in the College of Agricultural Engineering Sciences / University of Baghdad) for cryopreservation at -4 °C, then DNA extraction from blood using the kit of Extraction of DNA according to the instructions in the attached leaflet supplied by Geneaid Company. The genomic DNA's integrity was assessed using electrophoresis on agarose gels. The ANXA9 gene polymerase chain reaction (PCR) technique is focused on the major polymorphism observed in Intron 4 of the ANXA9 gene. The 675 bp segment Gene Bank accession number AY785286.1 observed in sheep genomic DNA was amplified using the matching primer pairs and the Annealing temperature 55.5°C at 30 cycle for 30 sec, according to the size of the fragments and kind of primers employed (forward and reverse). The details of the primer sequences are as follows:

F:5' CATTCCTGTGTGTCCGGTAC 3'

R: 5' TCATCTCAGACCTAACCACCA 3'

After the end of the polymerase reaction, the ANXA9 gene polymorphism was detected in sheep blood samples using a sequencing technique using the NCBI Blast software (by Nappo Corporation) and data program. In the genius program, the genotypes of ANXA9 were discovered by comparing the different sequences in nitrogen bases for the studied sheep to the wild sequence of the gene. The daily amount of milk for each sheep was measured using a cylinder in millimeters, once a month till the conclusion of the productive season. lambs are separated from ewes in the evening, and mothers are milked

in the morning. Milk components were analyzed once a month for each ewe during a three-month period. After weighing the milk, a sample was obtained in the morning and mixed well in clean plastic containers of 50 ml capacity with tight lids that were closed after collecting the sample and carried refrigerated to the laboratory to be examined in the milk components analyzer. (Milk analyzers Julie Z7), as it shows the percentages of protein, lactose, fat and solid non-fat [18]. The data was analyzed by used Statistical analysis system [19] program was used in the analysis of data to study the effect of the ANXA9 gene polymorphism on the studied traits by applying the general linear model (GLM), according to the below mentioned equation. The significant differences were compared between averages by Duncan [20] multiple range test with the application of least square means.

Statistical model: (Traits on ewes).

$Y_{ijk} = \mu + G_i + A_j + e_{ijk}$

Y_{ijk} = The observed value , μ = The Overall mean of trait ,G_i = The effect of gene polymorphism , A_j = The effect of ewe age , e_{ijk}= The random error.

Statistical model: (Traits on lambs).

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

Y_{ijklm} = The observed value , μ = The Overall mean of trait, G_i = The effect of gene polymorphism of dam , A_j = The effect of Age of dam at birth , S_k = The effect of Sex , T_l = The effect of type of birth , e_{ijklm}= The random error.

Chi-square (χ^2) test was used to significantly compare between the percentage (0.05 and 0.01 probability) in this study. Calculator of allele frequency of ANXA9 gene according of Hardy Weinberg's equilibrium [21].

Results and Discussion

PCR Amplification of Chromosome 1 Intron 4 for ANXA9 Gene

PCR technique amplification of genomic DNA. The results showed that the amplification fragment size of ANXA9 gene intron 4 was 675 bp, the PCR products were separated on 1.5-2% agarose gels and DNA ladder were (100 bp), all samples were amplified successfully, and a single band stained with Ethidium Bromide was obtained, as shown in the Figure (1).

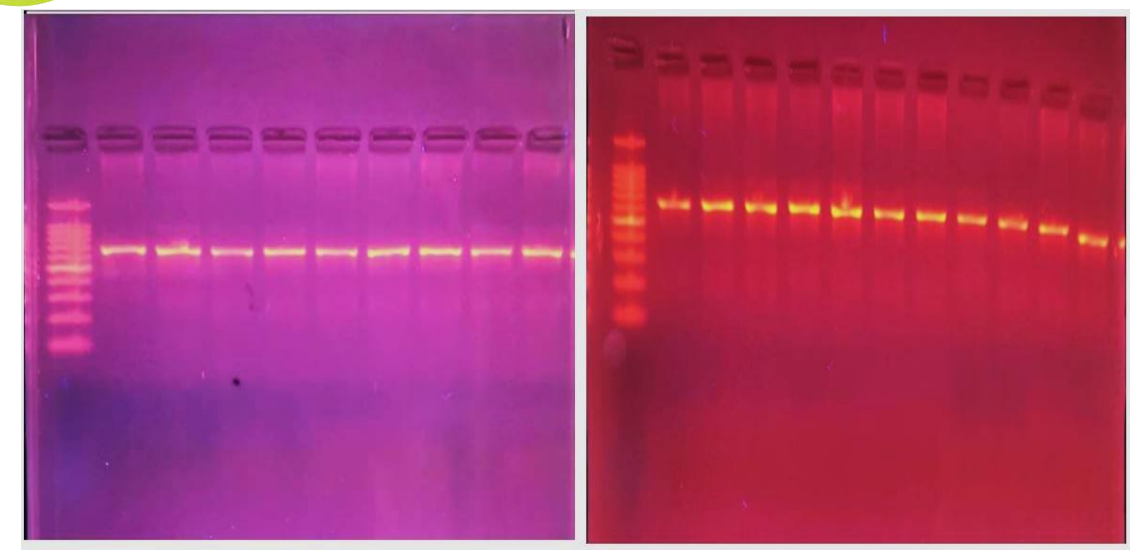

Figure (1): The PCR product band of intron 4 of ANXA9 gene with size 675 bp . The sequences of the nitrogenous bases of the ANXA9 gene

The nitrogen base sequencing technique was used, and the results revealed that the studied segment of the ANXA9 gene (675 base pairs) had in the target coding region of the ANXA9 gene, as three polymorphism appeared in this variation (T>G SNP) which are TT, TG and GG, as shown in Figure (2).

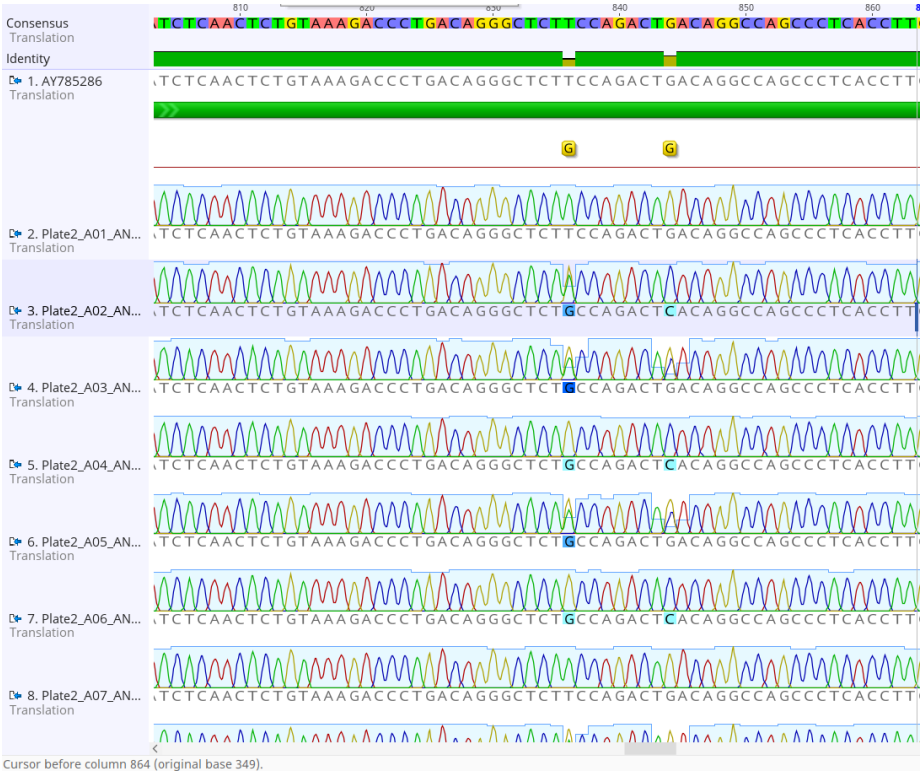

Figure (2): The site of the variants in the ANXA9 gene in Awassi sheep, The nitrogenous bases sequences of the ANXA9 gene

The number of polymorphism, their percentages, and the allelic frequency of the ANXA9 gene were shown in Table (1), three polymorphism appeared, and the percentage of TT, TG, and GG polymorphism were 51.92, 40.38 and 7.69 %, respectively, while the allelic frequency of the T and G alleles was 0.72 and 0.28, respectively.

Table (1): Distribution and allele frequency of ANXA9 gene /T>G SNP Polymorphism in sample study of Awassi sheep

ANXA9 gene /T>G SNP Polymorphism	Number	Percentage (%)
TT	27	51.92
TG	21	40.38
GG	4	7.69
Total	52	100%
Chi-Square (χ^2)	---	22.269 **
Allele	Frequency	
T	0.72	---
G	0.28	---
** (P≤0.01).		

Relationship of ANXA9 gene with Daily milk yield-DMY and Lactation period of SNP (T>G) Polymorphism

The results showed that significant differences (P≤0.05) in DMY (kg) between the TG, and GG polymorphism, it was 1128.65 ±22.30 and 973.75 ±42.67 (kg), respectively, But no significant differences between TT and TG polymorphism in DMY (kg), as well as, no significant differences among TT, TG and GG polymorphism in Lactation period (Table 2). Gutiérrez-Gil, Esteban-Blanco, Suarez-Vega and Arranz [22] reported that, the Milk composition, growth rate and body weight increase, is an economically important production-related feature in sheep, but its genetic foundation has not been studied as completely as that of cows. QTL or candidate gene research may help us understand the genetic foundation of milk composition and identify specific genotypes that impact it. Prior to the discovery of QTL for milk output in chromosomes 2, 3, 20.QTL for milk yield had been reported in several sheep populations but no meaningful associations had been proven. Several authors found QTL associated to protein content and yield in chromosomes 1, 2, 3, 5, and 6 in their investigations of several sheep breeds [23]. The ANXA9 gene in sheep was identified on chromosome 1 , within microsatellite markers INRA006 and AE57 , In this area the QTL for protein content in milk and milk production were also identified, it coding gene was shown to

be expressed in a variety of organs, such as the mammary gland. ANXA9 is a phospholipid and Ca^{2+} binding protein encoded by the ANXA9 gene. It is also engaged in cytoplasmic membrane transfer, which is essential in the mammary gland [24]. It has a molecular mass of 37 kDa and relates to the annexin family of Ca^{2+} and phospholipid-binding proteins [25].

Table (2): Relationship of ANXA9 gene /T>G SNP Polymorphism with Daily milk yield-DMY and Lactation period

ANXA9 gene /T>G SNP Polymorphism	Mean ± SE	
	DMY (kg)	Lactation period (day)
TT	1002.16 ±19.65 ab	118.37 ±8.94
TG	1128.65 ±22.30 a	123.09 ±11.51
GG	973.75 ±42.67 b	109.75 ±8.26
Level of Sig.	*	NS
Means having with the different letters in same column differed significantly. * (P≤0.05), NS: Non-Significant.		

Relationship of ANXA9 gene with milk composition of SNP (T>G) Polymorphism

The results appeared that there were significant differences (P≤0.05) between the different polymorphism in the percentage of milk fat, as the TT polymorphism (5.64 ±0.35) was significantly superior to the TG (4.41 ±0.39) but, no deferent with GG polymorphism (5.67 ±0.36), while milk lactose, milk protein and Sold not fat in milk, the results did not show any significant differences between the different polymorphism (Table 3)

Ogorevc, Kunej, Razpet and Dovc [26] reported that ANXA9 might be considered a potential gene for milk production traits as well as somatic cell count in cattle, because due to its similarity to QTL for these characteristics. Kulig, Kowalewska-Łuczak, Kmieć and Wojdak-Maksymiec [27] discovered that the ANXA9-A/G SNP substantially (P≤0.01) influenced somatic cell count in addition to protein and fat content in Jersey cattle, resulting in the selection for the ANXA9-A/G polymorphism to decrease somatic cell count together with increase protein and fat content, Therefore, preference for cattle with the ANXA9 A gene may contribute to lower somatic cell count, these findings might be used in genetic improvement for milk production attributes. While, Protein and fat content genetic variation suggests that selection for these traits may be beneficial.

Urrutia, Mendizabal, Alfonso, Soret, Insausti and Arana [28] stated that Because the biological hydrogenation process occurs in the rumen region, diet is the most important element in defining the fatty acid composition in bovine milk. Regrettably, the quantity of fatty acids in sheep milk exhibits low to intermediate genetic variation

(ranging from 0.01 to 0.47) for specific fatty acid [29]. As a result, diet and genetics have an important role in influencing the composition of milk fat [30].

Table (3): Relationship of ANXA9 gene /T>G SNP Polymorphism and milk composition

ANXA9 gene /T>G SNP Pol-ymorphism	Mean ± SE			
	Milk fat (%)	Milk lactose (%)	Milk protein (%)	Sold not fat in milk (%)
TT	5.64 ±0.35 a	5.16 ±0.10	4.92 ±0.13	10.85 ±0.16
TG	4.41 ±0.39 b	4.90 ±0.12	4.81 ±0.38	10.89 ±0.42
GG	5.67 ±0.36 a	4.89 ±0.03	4.82 ±0.12	10.51 ±0.16
Level of Sig.	*	NS	NS	NS
Means having with the different letters in same column differed significantly. * (P≤0.05), NS: Non-Significant.				

In light of the aforementioned results included in the current study on the relationship of target coding regions in the ANXA9 gene, the study found that the target coding region in the ANXA9 gene (T>G SNP) has a role in causing a significant variation in the percentage of milk fat of Awassi ewes.

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