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Original Research Article

Relationship between Polymorphism of the Lactoglobulin Gene Exon 2 and Productive Traits in Awassi Sheep

Razzaq Nasser Abed AL-Jubouri[1]*, Fouad Lazim Jasim ALkhuzai[2], Dheya D. Radhi[3], Al-Waith H. K[4], Riyadh Hamad Senkal[5]

[1]College of Biotechnology, Al-Qasim Green University, Iraq
[2]Southern Technical University Technical Institute, Shatra, Iraq
[3]Department of Animail production Technogies Shatra Technical Institute Southern Technical University, Iraq
[4]Department of Animal production College of Agriculture, University of Baghdad, Iraq
[5]Department of Animal production, Coll. Agric. Engin, Sci, University of Baghdad, Iraq

***Corresponding Author:** Razzaq Nasser Abed AL-Jubouri
College of Biotechnology, Al-Qasim Green University, Iraq

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Abstract: This study was carried out to examine the effect of changes in the β-lactoglobin EXON2 gene on the performance of Awassi ewes. The extracted 471-bp exon 2 gene of the β-lactoglobulin gene was utilized to determine the genotype of the gene that related to milk quantity and composition in the sample studied using the nucleotide sequence variations and their association. Most SNP1 alleles in the Kreshi ewes sample was (AG) genotype (56%), and the (GG) genotype (44%). An important impact ($p<0.05$) of SNP1 on milk lactose was observed in the (GG) genotype with the effect being (4.72±0.16) compared to (4.40±0.03) in the AG genotype. The other qualities that were tested did not demonstrate any major effects.

Keywords: β-Lactoglobulin, Exon 2, Genotypes, Milk Quantity, Awassi Ewes.

INTRODUCTION

Rearing domestic sheep as a source of meat, wool, and milk started around 11,000 years ago in Asia and the Middle East and is now done on a worldwide basis. It is estimated that there are more than 2200 million sheep and goats all over the world in total (Pulina *et al*., 2018; NSM, 2022). Among the 106 known species of sheep 20.8% are pure milk producers. Another largely untapped branch of the small ruminant industry is the dairy sheep industry (Djaout *et al*., 2022), Sheep milk can be converted into cheese and milk powder in addition to meat and wool, which is why there is a lot of potential of domestic and foreign businesses to make profits. The lack of documentation with respect to pedigrees and performances is one of the principal setbacks in enhancing the genetic characteristics of the flocks of small-scale farmers in developing livestock systems. The documentation is essential in genetic assessment and sound selection choice (Al-Anbari *et al.*, 2008; Gizaw *et al.*, 2022). Al-Anbari and Al-Samarai (2007), Al-Sarai and Al-Anabri (2019), and Naeemah and Al-Anbari (2023) all concur that genetic selection now can be used as an important instrument to enhance genes. There is a growing interest among the people in environmental and genetic betterment, interaction between the two and the continuously rising need of agricultural produce particularly animal produce in the world (Elia *et al*., 2005; Al-Anbari *et al.*, 2006). Small farmers have adopted subjective factors such as body morphology and the performance of the prospects to a large extent when selecting breeding stock. They also look at animal connections particularly along the dam line since majority of the farmers are aware of the location of their animals dams (Kugonza *et al*., 2012). The invention of the polymerase chain reaction (PCR) technology was a turning point that changed the course of the biological sciences and consequently other related disciplines (Bhat *et al*., 2022; Chen *et al*., 2022a). Many biologists have invested in this technology to examine and observe genetic mutations that take place in living beings, including Single Nucleotide Polymorphisms (SNPs). To direct selection, they rely on these mutations as genetic markers particularly low-genetic-value productive traits which are regulated by a set of sites known as quantitative trait loci (QTL) (Fadhil, 2019; Raza *et al*.,

2023; Wu *et al*., 2023). One can find out the markers of these QTL, estimate the phenotype variance of the traits that are to be improved initially, and construct selection programs with the help of these locations (Al-Anbari and Al-Samarai, 2007; Lorenz, 2012). As a result of one of these genes is PAEP which is the milk component of ruminants, beta-lactoglobulin (βLG). The PAEP gene of sheep contains seven exons located on chromosome three. The purpose of our research was to identify whether milk production of the Awassi ewes and the change in the Beta-lactoglobulin (βLG) gene have any relationship.

MATERIALS AND METHODS

We measured the quantity and the composition of milk at the College of Veterinary Medicine, Public Health Laboratory of Al-Qasim University. Although the genetic analysis was being conducted at the Biotechnology Laboratory of Al-Qasim green university, DNA sequencing was conducted at Macrogen, Korea, which is outside the Iraqi territory.

Research Using Live Animals

This was done using fifty Awassi ewes which had an age of between two and six years. They were in fine condition, had recently borne twins or one lamb and were lactating (not dry). The ewes were housed in semi-open quarters in order to house them.

Collecting Blood Samples

K3 EDTA anticoagulant was added to 5 ml of blood which had been collected in EDTA tube in each of the animals was drawn in the jugular vein. At the lab, the samples were put in a container and frozen at -18 0 C until DNA extraction could be done. Fifty milk samples were also collected among ewes which belonged to the Awassi breed. It was chilled in 60 ml plastic storage containers to preserve the nutrients of the milk. The milk contents were analyzed using a LactoFlash analyzer. With the help of polymerase chain reaction (PCR), an EXON2, the 471-base-pair band of the 471-base-pair gene of beta-lactoglobulin was obtained in the second expression region.

Primer Selection

The primer was selected based on the guidelines listed on Table (1) so as to molecularly identify and detect exon (2) of the 471-base-pair band of the 471-base-pair 967-base gene of -lactoglobulin.

Table 1. Shows the primer sequence prepared by IDT Integrated DNA Technologies, Canada: β-lactoglobulin

Gene abbreviation	Sequence	Product size	Source
β-lactoglobulin EXON2	(F) CCCAAGATCCAAATGTTGCT		
	CGCCGGGTACCAGTAAACTC (R)	471bp	

(Rashaydeh et al., 2020)

Specific PCR (Polymerase Chain Reaction) Primers

Exone 2 2 -lactoglobulin gene was molecularly identified by polymerase chain reaction (PCR) using the materials indicated in Table 2. The 25 µL samples were then placed in the PCR machine using the reaction parameters of the segment to be amplified (Exone 6). The tubes were transferred into the PCR machine after the ingredients were mixed using the vortex mixer. The PCR conditions were established according to the instructions of the table.

Table 2. PCR reaction components for JAK2 Exon 6 (Promega Master Mix, USA)

Component	Volume (µL)
Master Mix	12.5
DNA Template	3.0
Forward Primer	1.0
Reverse Primer	1.0
Distilled Water	7.5
Total Volume	**25.0**

Table 3. Thermal cycling protocol used for JAK2 gene amplification (Oster *et al.*, 2023)

Step	Temperature (°C)	Time	No. of Cycles
Initial Denaturation	95°C	5 minutes	1
Denaturation	95°C	30 seconds	
Annealing	58°C	45 seconds	35
Extension	72°C	45 seconds	
Final Extension	72°C	5 minutes	1

Nucleotide sequence of the gene of β-lactoglobulin was examined. To analyze the sequencing findings, the NCBI database was searched to analyze the sequence. The existence of the JAK2 gene was confirmed using Geneious Prime, BioEdit, and MEGA version 12 (Kearse *et al*., 2012; Kumar *et al*., 2018).

Data Analysis by Statistic

The data were analysed using the SAS program (version 30) to ascertain the impact of the 2 -lactoglobulin gene (Exon 2) on the attributes that were assessed. A chi-square test (2) was used to test the genotypic frequency distributions of each SNP within the JAK2 Exon 6 region and Duncan Multiple Range Test to test the variation between means.

RESULT AND DISCUSSION

DNA Extraction

DNA was extracted using a commercial DNA extraction kit in an attempt to isolate the JAK2 gene (Exon 6). The effectiveness of extraction technique was checked through electrophoresis of all the materials on an agarose gel.

Increase in the Amplification of Target Region of the 2 -Lactoglobulin Exon

The target region of the 2nd exon of 2-lambda-lactoglobulin was amplified by the Polymerase Chain Reaction (PCR) technique. The DNA samples, supplementary primers, and PCR master mix underwent the reaction as described above in accordance with the protocols described in the Materials and Methods section. Then, the PCR products underwent gel electrophoresis on a 1.5% agarose gel. A molecular size marker in the form of a 100 bp DNA ladder was used to confirm that the target region (~ 280 bp) has been amplified (Figure 1).

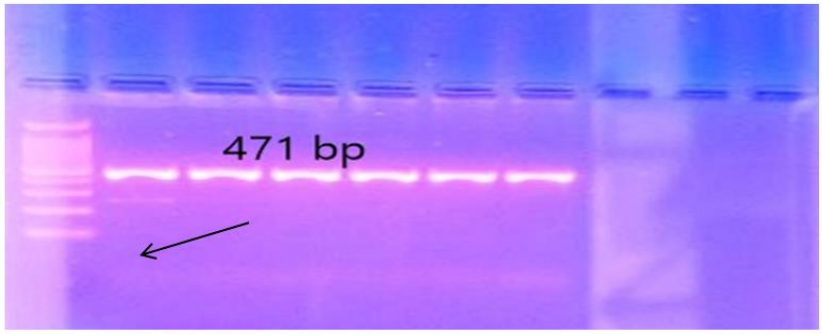

Figure 1. Amplification of the target region of the JAK2 gene (Exon 6) (A>G)(NC_056055.1) visualized on a 1.5% agarose gel.

Allelic Frequency and Genotypes

Table (3) presents the genotype percentages of the 2 exon (471 base pairs) 5 of the 5-base 2 -lactoglobulin gene. Two genotypes were exhibited; GG and AG, and there was one mutation. This mutation led to an exchange of alleles as in the Figure (2), G was more common than A.

Table 3. Genotypes and Allelic Frequency of the β-lactoglobulin Gene exon2

SNPs Allele	Genotype Frequency	No			%
SNP1	GG	22	44	G	0.44
	AG	28	56	A	0.56
	المجموع	50	%100		
χ^2	----	22.54**	--		---
: عالي المعنوية.					

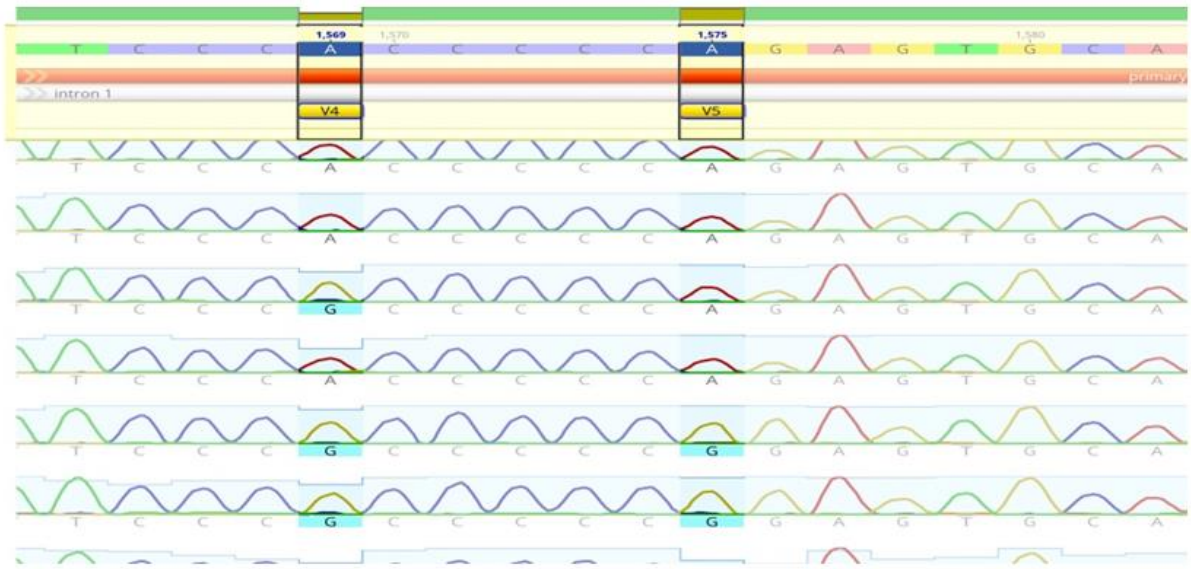

Figure 2. Shows the location of the mutation in the β-lactoglobulin gene exon2

Effects of 2 SNP1 Gene Variants of 2 2 -Lactoglobulin Exon 2 on Quantity and Quality of Milk of Awassi Ewes

Table (4) that shows the milk quantity and components trait in Awassi ewes during the first milking has a greater advantage of AG genotype over the GG genotype on the basis of milk lactose content at a probability of (P≤0.05) in comparison to GG genotype. The other attributes which were studied did not portray any significant differences. They agree with the work of (Guo *et al*., 2022) who concluded that milk production was greater in animals with GA genotypes of this gene than in homozygous genotypes. Similarly (Othman, *et al*., 2021) experimentally concluded that GA genotype had significant associations with increased milk production and improved milk constituents of Palestinian Awassi sheep. Ewes with GA genotype showed a very high average protein percentage (3.21 0.50) compared to the AA (2.48 0.10) and GG (2.32 0.10) and this indicates that GA genotype is very useful in maintaining high milk quality especially in nutritional value of protein.

Table 4. Effect β-lactoglobulin Exon 2 SNP1 Genotypes on Milk Yield and Composition in Karashi Ewes

Trait	Genotype AG	Genotype GG	Significance
Milk Yield	1760.71 ± 38.34	1702.27± 30.41	N.S
Fat % (F)	3.87 ± 0.41	4.69 ± 0.53	N.S
Total Solids %	10.75 ± 0.22	10.68±0.28	N.S
Protein %	5.48 ± 0.18	5.36±0.24	N.S
Lactose %	4.40 ± 0.03	4.72 ± 0.16	*

**Note: NS = Not Significant, * = Significant at (P ≤ 0.05), ** = Significant at (P ≤ 0.01).*

The Effect of SNP1 Gene Variants (Exon 2 -Lactoglobulin Exon 2) on the Milk Produced by the Awassi Ewes in the Second Pull and its Composition

Table (5) indicates that despite the few characteristics that were near significant, no significant differences were observed in the probability level (P=0.05) between the 2 Exon 2 genotype of 8 -lactoglobulin genes in the studied traits. This is founded on information on milk composition and quantity on Awassi ewes. This is in agreement with what was discovered in (Razaq *et al*., 2020).

Table 5. Effect β-lactoglobulin Exon 2 SNP1 Genotypes on Milk Yield and Composition in Karashi Ewes

Trait	Genotype AG	Genotype GG	Significance
Milk Yield	2062.50± 43.46	1990.91± 44.09	N.S
Fat % (F)	4.45± 0.50	5.30± 0.57	N.S
Total Solids %	10.59± 0.23	10.66± 0.30	N.S
Protein %	5.38± 0.18	5.14± 0.24	N.S
Lactose %	4.40± 0.03	4.67± 0.16	N.S

**Note: NS = Not Significant, * = Significant at (P ≤ 0.05), ** = Significant at (P ≤ 0.01).*

Effects of Genetic Modulations of 3 SNP1 2 Exon of 2 -Lactoglobulin on Milk Quantity and Composition in Awassi Ewes (Third Draw)

As indicated in Table (6) that presents the data on milk volume and composition of Awassi ewes at draw 3, no significant differences were found in the β-lactoglobulin Exon 2 gene genotypes of the study traits though some of them were near the level of significance. This was established at the level of probability (P= 0.05). Based on such findings, we discover (Hader *et al*., 2021).

Table 6. Effect β-lactoglobulin Exon 2 SNP1 Genotypes on Milk Yield and Composition in Karashi Ewes

Trait	Genotype AG	Genotype GG	Significance
Milk Yield	2348.21± 53.04	2215.91± 48.05	N.S
Fat % (F)	7.77± 0.29	6.94± 0.31	N.S
Total Solids %	10.85± 0.14	10.91± 0.16	N.S
Protein %	4.04± 0.05	4.03± 0.05	N.S
Lactose %	5.94± 0.09	6.02± 0.08	N.S

**Note: NS = Not Significant, * = Significant at (P ≤ 0.05), ** = Significant at (P ≤ 0.01).*

CONCLUSIONS

The SNP1 genotype of the 2 Exon locus of β-lactoglobulin SNP1 at G G encourage an elevation in the content of milk lactose as per the findings of the study. This hopefully has been an indication of a good genetic marker in future breeding endeavors. Mutations had no considerable influence on the production and composition of the milk, and such parameters as fat and protein content or milk output did not considerably differ as well. These results could be attributed to the effects of environmental factors and the polygenic nature of some of the traits. Such findings have emphasized the need to investigate a large number of mutations by techniques such as genome-wide association studies (GWAS) and the need to scale up research to incorporate more genomic loci in order that local Awassi sheep can have their genetic effect better determined.

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