ARTICLE / INVESTIGACIÓN

Effect of the prolactin gene polymorphism on quantity and quality of milk in Iraqi goats

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DOI. 10.21931/RB/2022.07.04.22

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Abstract: Domestic goats in Iraq are one of the oldest domesticated animals in Mesopotamia. Their adaptation characterizes them to harsh environmental conditions such as heat in the summer and cold in the winter. Still, they suffer from neglect and the lack of genetic research that would improve the production of this animal, which is considered a multi-production (milk production, meat production, hair production). And because the prolactin gene is crucial, the current study focused on how the prolactin gene (PRL) affects milk yield and its composition. We obtained three genotypes of the prolactin gene exon 5: AA, AB, and BB. The hybrid genotype AB had the highest daily milk yield in domestic goats, although the recessive type BB had a more significant fat percentage. The genetic approach is advised if breeding aims to enhance milk output daily. So select hybrid genotype AB to exon 5 for the (PRL) gene.

Key words: Domestic Iraqi goats, prolactin gene, milk production.

Introduction

Livestock output in Iraq typically falls behind agricultural production in terms of development, management standards, and husbandry; crop farming and raising livestock have not always been combined¹. The native goat breeds in Iraq are extensively distributed throughout the country and bred with sheep as a mixed flock. They are noted for their determination to endure the challenging conditions of poor pastures and high disease resistance. Typically, goats graze intensively on natural grassland and agricultural wastes with sheep for more than six months out of the year. There is relatively little forage produced². Goats of the Iraqi strain are all different shades of white, black, and brown. Each color spreads with the other colors mentioned in varied ratios². The native goats in Iraq have a population of roughly 1.3 million. It is dispersed throughout the nation, with I2.5, 44.2, and 43.3 percent being in the country's southern, central, and northern regions, respectively¹.

Production of meat and milk is the primary goal of goat farming, but hair production is still a secondary goal. Through utilizing advancements in genetic and biological sciences and identifying numerous genetic markers that were strongly correlated with the phenotype variance of significant traits like milk production, meat production, reproductive traits, and behavioral performance, numerous attempts were made to improve native goat performance³. Prolactin (PRL) is a protein hormone that controls breastfeeding and reproductive processes in animals^{4,5}. And is mainly released by eosino-phils in the anterior pituitary gland. Prolactin regulates the differentiation and proliferation of mammary gland cells to encourage milk production throughout the development of animal mammary glands⁶.

Additionally, PRL regulates milk's protein and lipid concentrations and the production of lactose, among other critical functions⁷. Many studies have found that PRL polymorphisms are linked to wool or cashmere traits in goats and sheep⁸. Others investigated the relationship between PRL polymorphism and dairy traits such as benefit yield and protein milk yield in cattle breeds⁹. Furthermore, many researchers have suggested that the PRL gene improves prolificacy in various sheep breeds. Since there are few studies on the prolactin gene in local Iraqi goats, our research chose to shed light on how the polymorphism of the prolactin gene in local goats relates to milk production and its constituent parts.

Materials and methods

The 50 female goat samples included in the current investigations are from Iraq. Fifty female goats, mostly from AI-Mahawil, were found in numerous commercial farms in Babylon City between October 1, 2020, and June 1, 2021. The animals were raised in open fields and fed a concentrated diet (450–550 grams per head per day. Hand milking involves manually collecting the milk from the ewes during the preweaning stage once a week (until 115 days). The milk is then kept in ice until it is transported to the lab to be calculated, and its various components are examined in the morning and evening. After milk filtration, a public health lab in Baghdad utilized Lacto flash solution to evaluate the milk's composition.

To determine the genetic structures of the studied gene segment, each female goat had approximately 5 ml of blood drawn from the jugular vein, which was kept in a sterile container at 4°C for DNA extraction^{2,16}. The following PCR conditions were used: 3 l of genomic DNA, primer, 10 mM Tris-HCI (pH 9.0), 30 mM KCI, 250 M of each dNTP ("dATP,

Received: 20 July 2022 / Accepted: 15 October 2022 / Published: 15 November 2022

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Citation: Alsarai T M. Effect of the prolactin gene polymorphism on quantity and quality of milk in Iraqi goats. Revis Bionatura 2022;7(4) 22. http://dx.doi.org/10.21931/RB/2022.07.04.22

dGTP, dTTP, and dCTP"), 1 U Top DNA 2 polymerase, and 1.5 mM MgCl (BioNeer Company, Korea). The generated DNA fragment was amplified using one primer from Integrated DNA Technologies. As mentioned below about the details of the primer sequence for exon 511 :

F: ATTCCTGGAGCCAAAGAG R: TGTGGGCTTAGCAGTTGT Product Length: 655pb Restriction Enzyme: Eco 241

As a result, the study used restriction enzyme (2U/ Rsal I) for two hours at 38°C to reveal the DNA fragment sizes between experimental ewes. Restriction fragment length polymorphism (RFLP) identifies specific restriction enzymes that show a pattern difference between the DNA fragment sizes in individual organisms¹⁰. The study used gel electrophoresis and the restriction enzyme to digest the product with roughly 1.5% from gel agars. Then it estimated the frequency of alleles and genotype of the prolactin gene in female goats.

Statistical Analysis

The data, according to the SAS application¹¹.

Were examined.

 $Yij = \mu + \alpha i + eij$

Where: Where: μ : is an overall means, α : the genotype of the prolactin gene.

eij: is a random error, and genes (AA, AB, and BB) and their effect and eij: is an unexpected error.

Calculations were done using the genotype and allele frequencies Formula¹².

Gene frequency = 2D + H/2N

If H is the number of animals with heterozygosis, D is the number of homozygous animals for specific alleles

N: the total number of animals. Chi-square (χ) test was used to predict the essential variations between individuals $X = 2 \Sigma$ (Observed No. – Expected No.)2/ Expected No.

GLM, a general linear model, was utilized in the study, and Duncan's numerous ranges Test¹³.

To predict the significant difference between groups.

Results

Milk production and composition are examples of some quantitative traits loci (QTL) in Iraqi goats. Our study was about the genetic pattern of exon 5 for (PRL) gene and its relationship to the Daily productive performance of the milk production recipe and its milk components.

Genetic polymorphism

Our data analysis shows that RFLP-PCR is a valuable approach for estimating the genetic variability of Iraqi goats. In an Iraqi goat, a 420 bp PRL gene fragment was amplified using a specific primer (Fig 1).

The genetic polymorphism and allele frequency for the PRL gene were measured in the current investigation. As shown in table(1), the percentages of the AA, AB, and BB genotypes were each (46, 34, and 20)%, respectively.

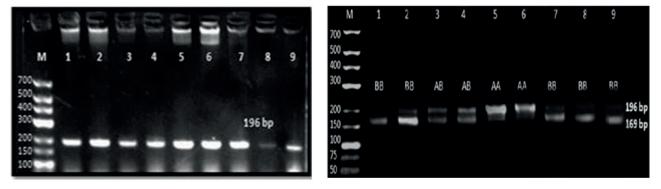
The relationship of genotypes to milk production and its components

In our study, when we compared the AB, AA and BB genotypes in Iraqi goats of the PRL gene, we found the height level of fat composition connected to the BB genotype was 5.16, and the lowest level was 3.81. While the proportion of proteins related to the BB genotype was 2.66, and the balance to the AB genotype was 1.28, respectively. Moreover, as explained in the table, the high ratio of solids nonfat (SNF) connected BB was 7.10, and the lowest with AB was 3.47; table (2).

Discussion

Fifty blood samples in our current study using PCR-RFLP methods, the results of genetic polymorphism were three genotypes (AA, AB, and BB. The percentages of their genotypes, as given in table (2), were each (46, 34, and 20)%, respectively. Also, the percentages of allele frequency were 0.36 for A and 0.37 for B. This is different from the study on Egyptian goats, as the ratio of hybrids is higher than other genotypes¹⁴. But it agrees with what was stated in the study about Chinese goats since the AA type is the most numerous⁸. It is possible to attribute the results to the in-field breeding strategy.

This gene was selected due to its direct contribution to milk synthesis, secretion maintenance, and mammary gland growth and development^{7,15}. However, there is little evidence of the relationship between this marker and milk yield and composition in Iraqi dairy goats. As shown in table(2), our study discovered that the height level of fat composition associated with the BB genotype was 5.16, and the lowest level was 3.81. Furthermore, 2.66 percent of proteins were linked to the BB genotype, while 1.28 percent were related to the AB genotype. Additionally, as shown in the table, BB and AB had the highest and lowest ratios of solids nonfat



(a)

 (\mathbf{b})

Figure 1. (a): Agarose gel electrophoresis of PRL-PCR fragment (196 bp). Lane M, 50 bp DNA ladder (b): Agarose gel electrophoresis of PRL- Eco24I/PCR-RFLP fragments. Lane M, 50 bp DNA ladder, lanes (5, 6) Genotype AA (196 bp), lanes (3, 4) Genotype AB (169 to 196 bp) and lanes (1, 2, 7, 8, 9) Genotype BB (169 bp).

Genotype	No	Percentage (%)	
AA	23	46.00	
AB	17	34.00	
BB	10	20.00	•
Allele	Frequency		- ·
A	0.36		
Т	0.37		
	** (P≤0.01).	I	

Table 1. Genetic polymorphism and allele frequencyfor the PRL gene.

	AA	AB	BB
Daily milk yield (gm)	420.56 ±26.20	601.22 ±30.22	400.22 ±22.23
Fat	3.81 ± 0.01	$4.27\pm\!\!0.02$	$5.16{\scriptstyle\pm0.10}$
Protein	$2.22\pm\!0.03$	1.28 ± 0.01	$2.66\pm\!0.22$
SNF	5.99 ± 0.11	3.47 ±0.12	7.10 ±0.21
Relative density	1.014 ± 0.21	1.00 ± 0.01	$1.017\pm\!0.23$
Freezing point	-0.89 ± 0.03	-0.22 ±0.13	-0.46 ±0.04

Table 2. The relationship of PRL genotypes to milk production and its components in Iraqi domestic goats.

(SNF), respectively, at 7.10 and 3.47; some studies confirm the importance of paying attention to this trait because it is linked to health benefits such as bone mineral density. It also reduces the risk of developing type 2 diabetes and coronary heart disease¹⁶; our results contrast the study which found that goats with the dominant Model AA of the exon five segment for prolactin gene produced the most milk daily¹⁵. Also, we found that the relative density was higher in the BB genotype in this study and in Table 2, which could be due to the correlation of density with the fat percentage in milk¹⁷, as the mentioned genotype has the highest rate of fat. Furthermore,

Our findings show that when this piece of the prolactin gene is studied, it becomes clear that if we want to select based on milk production, we choose the hybrid genotype AB. I hypothesize that the strength of the hybrid for this trait may affect the output level. Still, if we want to select based on fat and SNF proportion, we choose animals with the genotype BB that is the highest in these traits.

Conclusions

Goats are one of Mesopotamia's oldest domesticated animals. They are distinguished by their ability to adapt to harsh environmental conditions, such as heat in the summer and cold in the winter. Domestic goats in Iraq are neglected, and genetic research is lacking to improve their production. The prolactin gene (PRL) is a potential candidate gene for goat milk traits in marker-assisted selection. As a result, this research aimed to identify PRL gene polymorphism and its relationship to milk traits. So, if the goal of breeding is to increase milk output daily, we recommend for the (PRL) gene, choose hybrid genotype AB of exon 5. The hybrid genotype AB had the highest daily milk yield in domestic goats. However, if we want to select fat and SNF proportion, we choose animals with the genotype BB, which is the highest in these traits.

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