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Article

Relationship Polymorphism of STAT5A Gene in Performance Production of Local Goats

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ABSTRACT

The study was conducted on a sample of 30 local goats in Diyala governorate / Canaan district in one of the goat breeders' fields in the region for the period from 13/11/2022 to 1/5/2022, with aim of determining the genotype of the gene STAT5A EXON-7 and Its relationship to milk production (daily milk production-DMP and total milk production-TMP) and growth traits (birth weight-BWT, weaning weight-WWT and total weight gain-TWG). The analysis showed three-point mutations (CC=18 CT=12 C47064T AA=17 AT=13 A47088T and GG=15 GA=15 G47162A). There was no significant effect of the genotypes of the three mutations on all studied traits DMP, TMP, BWT, WWT, TWG, and body dimensions of the newborn at birth and weaning, body dimensions of the newborn at birth and weaning.

Keywords: Local goats, milk production, gene STAT5A EXON-7.

INTRODUCTION

Interest in raising and improving goats to benefit from milk and meat has recently begun, and goat milk is considered distinctive and healthy, especially for people who suffer from severe allergies [1, 2]. Because of the increasing population numbers around the world, the demand for animal products has increased, which prompted researchers to find ways to improve the productivity of farm animals, including goats, and this was evident in many different studies [3]. use of molecular genetics techniques has recently led to the discovery of genes that affect mainly and directly in many important productive and economic traits, as well as recognizing the effect of those genes by knowing the quantitative trait loci [4]. Therefore, genetic markers were relied upon. In selection programs because, they are more accurate than phenotypic and biochemical markers [5]. Many transcription factor binding sites in the region close to the catalytic regions (promoter) have an essential effect on the transcription process, such as Nf1, CIEBP, STAT5A, and GR [6], and the STAT5A gene plays an important role in many physiological processes. , as it is related to multiple traits such as the viability of fetuses and milk production traits [7], and the STAT5A gene is considered a mammary gland factor (Mammary Gland Factor-MGF), so it participates in the development of the mammary gland and is key in signaling the hormone prolactin (PRL) in addition to the activity of reproducing milk protein genes [8]. This gene is a transcription factor for the milk protein gene (K-casin) due to its relationship to milk production and mammary gland traits [9].

Because of the relationship of the STAT5A gene in milk production and growth traits in goats, as well as its sizeable genetic morphology, the study highlighted:

1. Determination of the genotypes of the local goats bred in Iraq based on the frequency and ratios of the genotypes of the STAT5A EXON-7 gene under study.

2. Studying the relationship of genetic polymorphism of STAT5A EXON-7 gene to animal performance in growth traits, milk production traits and its components.

MATERIALS AND METHODS

Used in the experiment are 30 local Iraqi goats. Molecular genetic analyses were carried out in the Biotechnology Laboratory of the Department of Animal Production / College of Agriculture / University of Diyala for the period from 2/1/2022 to 1/3/2022 to separate the genetic material (DNA) and conduct electrophoresis Polymerase chain reaction (PCR) analysis of STAT5A EXON-7 gene. The data on milk production were collected weekly for the morning circuit, and the newborn's birth weight was taken 12 hours after birth. The weaning weight was weighed after three months of birth, and the total weight gain was calculated through the weaning weight minus the birth weight, while the body dimensions of the newborns were measured. At birth and weaning according to method [10], blood samples were drawn from the jugular vein 2.5 ml using 5 ml test tubes containing Diamine Tetra acidic acid Ethylene (E.D.T.A.). After withdrawal, the blood samples were frozen at frozen temperature (-18).) It is used in the extraction of genetic material (DNA). Kit FAVORGEN is of Taiwanese origin.

Primer Seq-	Primer Name	Amplicon size (pb)	name gene	Annealing temp C°
F- CTGCAGGGCTGTTCTGAGAG	Primer–F	215 pb	STAT5A EXON 7	64°
R-TGGTACCAGGACTGTAGCACAT	Primer-R			

Table 1: Primer used with a length of 215 N bases of STAT5A EXON-7 gene in local goats.

After that, the studied segment of the STAT5A EXON-7 gene was detected using the polymerase chain reaction (PCR) technology. After the reaction was completed, the product of the polymerase reaction was migrated to ensure the presence of PCR products using the same method of preparing the agarose gel in the DNA migration, as DNA with a known molecular weight was loaded. (100-1500 nitrogen bases) in the first hole of the gel template and then loading the PCR product by 5 microliters into the pits of the gel template at (70V for 90 minutes) to be seen by the UV Light Transilluminator and photographing these beams. With a special camera, the packages appear colored orange ethidium bromide.

After electrophoresis, the PCR product, in a volume of 20 microliters, was sent to the Korean company Micro Gene Corporation. After obtaining the results by e-mail, the Genious Software was used to analyze the results on the global website of Genebank, www.ncbi.nlm.nih.go. The nucleotide sequence profile was used to determine the presence or absence of the mutation, and the curve profile to determine the phenotypic polymorphism of the three-point mutations of the STAT5A EXON-7 gene. The data were statistically analyzed using the statistical program SAS (Statistical Analysis System) [11] to study the relationship between the genetic structures of the STAT5A EXON-7 gene in milk production and growth traits for newborns. The test for significant differences between the means was carried out using Duncan's polynomial test [12].

$Yijklm = \mu + Tj + Sk + Ml + eijklm$

Since:

Yijklm: watch value m.

μ: the general average of the adjective.

Gi = the effect of the polymorphism of the STST5A gene on the studied traits.

Tj: the effect of birth type j (single, twin).
Sk: the effect of the gender of the newborn k (male, female).
Ml: The effect of the month of birth (November, December, January, February).
eikon: the random error that is usually and independently distributed with a mean of zero and a variance of σ^2e

RESULTS AND DISCUSSION

The current research detected point mutations in the studied sample of Region of STAT5A EXON-7 gene consisting of 215 bp. When analyzing the sequencing, three-point mutations, C47064T A47088T and G47162A, were detected with two genotypes (CC, CT) (AA.AT) and (GG, AG), respectively.

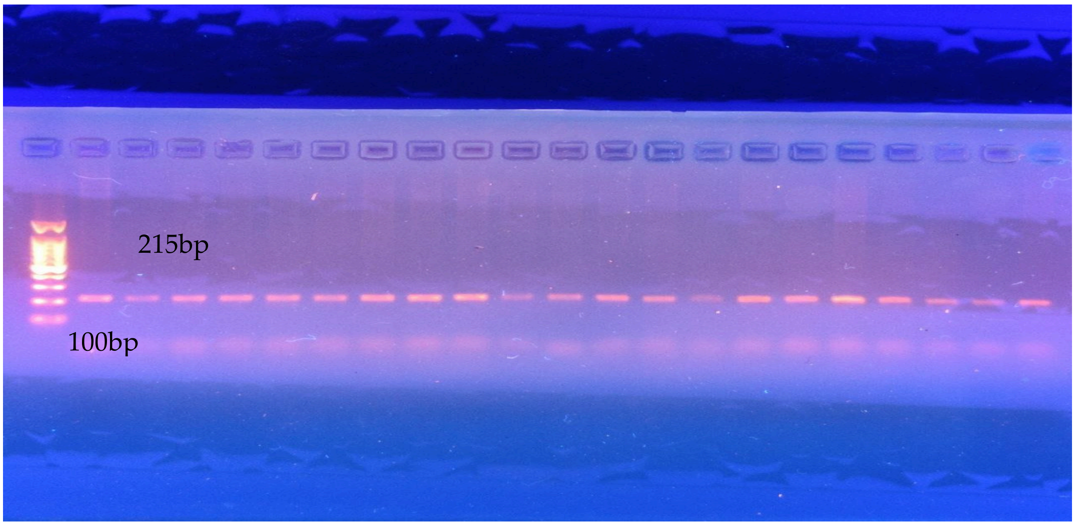

Figure 1: Shows the electrophoresis of the studied segment of the STAT5A EXON7 gene.

Were detected with two genotypes: the dominant wild CC and the hybrid CT. The frequency of the wild allele C is 0.80, While the mutant allele T is 0.20 (A47088T) with two genotypes, dominant wild AA and hybrid AT, the repeat of the wild allele A is 0.78 while the mutant allele T of 0.22, (G47162A) with two genotypes dominant wild GG and hybrid GA, the frequency of the wild allele G is 0.75 While the mutant allele C was 0.25 as shown in Table (1). A significant superiority of the chi-square value ($P \leq 0.01$) is observed for all mutations (C47064T) = 0.022 (A47088T) = 0.09 (G47162A) = 0.048.

mutations	genotypes	no.	percentage	observed	expected	chi-square value	allelic frequency
C47064T	CC	18	60	0.60	P^2=0.64	Total(observed-expected)^2/expected 0.022= p≤0.01	**C=0.80** **T=0.20**
	CT	12	40	0.40	2pq=0.32		
A47088T	AA	17	56.7	0.56	P^2=0.604	Total(observed-expected)^2/expected 0.09= p≤0.01	**A=0.78** **T=0.22**
	AT	13	43.3	0.43	2pq=0.343		

G47162A	GG	15	50	0.50	P^2=0.562	Total(observed-expected)^2/expected 0.048= $p \leq 0.01$	**G=0.75** **C=0.25**
	GC	15	50	0.50	2pq=0.375		

Table 2: Number, percentages of genotypes and allelic frequency of the studied mutations in the STAT5A EXON-7 gene.

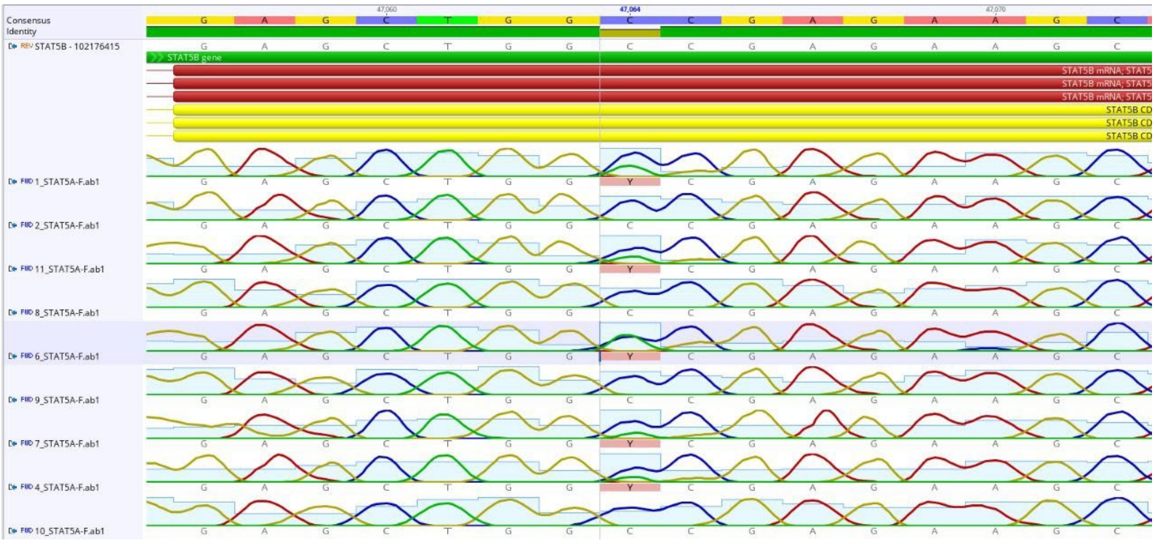

Figure 2: Shows the location of the mutation (C47064T) in the STAT5A gene EXON-7.

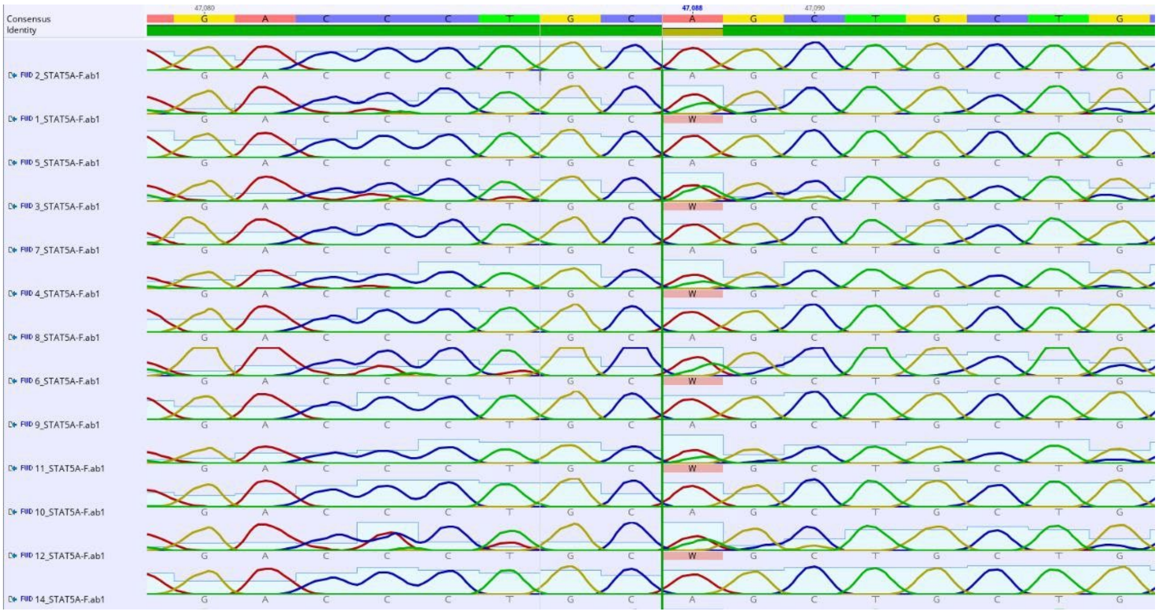

Figure 3: Shows the location of the mutation (C47088T) in the STAT5A gene EXON-7.

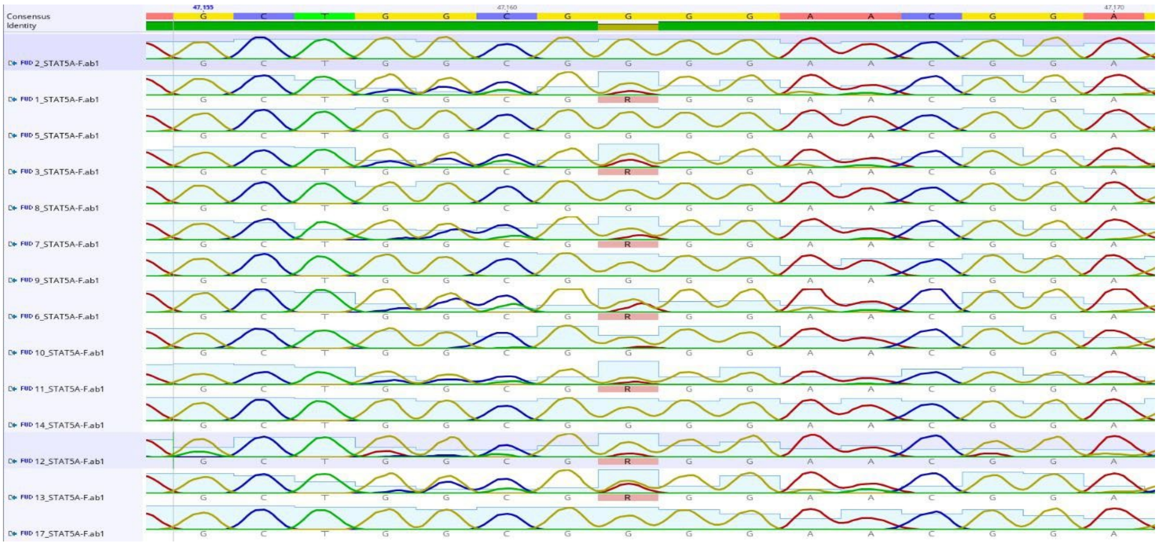

Figure 4: Shows the location of the mutation (C47062T) in the STAT5A gene EXON-7.

The results of the statistical analysis in Tables 2,3,4,5 and 6 showed that there was no significant effect of the point mutation (C47064T), (A47088T) and (G47162A) on Daily and total milk production, growth traits (birth weight, weaning weight and total weight gain) and dimensions at birth and weaning.

total milk production kg/season	**daily milk production g/day**	**No. 30**	**trait**
	C47064T		
2.61 ± 28.06	33.55 ± 359.80	18	**CC**
2.64 ± 26.15	33.93 ± 335.30	12	**CT**
	A47088T		
2.65 ± 26.43	34.03 ± 338.86	17	**AA**
2.65 ± 28.43	34.06 ± 364.56	13	**AT**
	G47162A		
2.96 ± 27.17	37.97 ± 348.39	15	**GG**
2.39 ± 27.42	30.70 ± 351.61	15	**GA**
NS	NS		**level significant**

Table 3: Shows the effect of genotypes in milk production for the studied point mutants.

total weight gain kg	**weaning weight kg**	**birth weight kg**	**no. 30**	**trait**
		C47064T		
1.11±11.54	1.12±14.43	0.17±2.89	18	**CC**
1.51±10.96	1.45±13.91	0.16±2.95	12	**CT**
		A47088T		
1.10±10.72	1.10±13.66	0.18±2.94	17	**AA**
1.27±12.08	1.43±14.95	0.13±2.87	13	**AT**
		G47162A		
1.23±10.90	1.24±13.89	0.18±2.98	15	**GG**
1.30±11.71	1.26±14.56	0.16±2.84	15	**GA**

NS	NS	NS		**level significant**

Table 4: Shows the effect of genotypes on birth weight, weaning weight and total weight gain.

body length cm	**back height cm**	**front height cm**	**chest circumference**	**abdominal circumference**	**no. 30**	**trait**
			C47064T			
0.57±24.27	0.97±36.61	0.79±35.22	0.80±33.83	0.94±35.0	18	**CC**
0.45±23.08	0.80±36.08	0.79±34.33	0.54±33.58	0.74±35.0	12	**CT**
			A47088T			
0.61±24.17	1.03±36.58	0.83±35.17	0.83±33.64	1.00±35.05	17	**AA**
0.41±23.30	0.74±36.15	0.73±34.46	0.55±33.84	0.69±34.92	13	**AT**
			G47162A			
0.68±24.40	1.10±36.86	0.93±35.33	0.89±33.86	1.06±35.20	15	**GG**
0.36±23.20	0.73±35.93	0.64±34.40	0.58±33.60	0.71±34.80	15	**GA**
NS	NS	NS	NS	NS		**level significant**

Table 5: Shows the effect of genotypes on the body dimensions of lambs at birth.

body length cm	**back height cm**	**front height cm**	**chest circumference cm**	**abdominal circumference**	**no. 30**	**trait**
			C47064T			
0.6±35.389	1.20±55.66	1.59±53.88	1.16±57.38	1.90±61.66	18	**CC**
0.9±36.088	1.64±56.16	2.30±53.08	1.40±56.75	1.96±62.41	12	**CT**
			A47088T			
0.6±34.820	1.26±55.17	1.56±52.82	1.17±56.94	1.86±60.64	17	**AA**
0.9±36.769	1.49±56.76	2.25±45.53	1.38±57.38	1.96±63.69	13	**AT**
			G47162A			
0.6±34.937	1.41±55.46	1.76±52.93	1.31±57.06	2.12±60.66	15	**GG**
0.8±36.409	1.33±56.26	1.96±45.20	1.21±57.20	1.71±36.26	15	**GA**
NS	NS	NS	NS	NS		**level significant**

Table 6: Shows the effect of genotypes on the body dimensions of lambs at weaning.

CONCLUSIONS

The results of this study showed the presence of three mutations within the studied region of the seventh exon: the first mutation at the C47064T site resulting from the transformation of the base C to T, the second at the A47088T site resulting from the transformation of the base A to T, and the third mutation G47162A resulting from the transformation of the G base to A. There was no significant effect of the studied mutations on milk production and growth characteristics.

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