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Research Article

The effects of SNPs between exons 6-9 in the STAT5A gene on milk yield in Anatolian buffalo

Emsal İBİŞ[1,a,✉] , Metin ERDOĞAN[2,b]

[1]Kütahya Health Science University, Gediz Health Services Vocational School, Department of Medical Services and Techniques, Kütahya, Türkiye;
[2]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Medical Biology and Genetics, Afyonkarahisar, Türkiye.

[a]ORCID: 0000-0001-8006-2954; [b]ORCID: 0000-0003-0975-1989.

ABSTRACT

The Signal Transducer and Activator of Transcription 5A (STAT5A) gene plays a crucial role in the development of the mammary gland and milk production by mediating prolactin (PRL) signaling. These functions make STAT5A a promising candidate gene for further research, especially in milk-producing animals. The aim of this study was to determine the relationship between polymorphisms in the 1720 bp segment spanning exons 6-9 of the STAT5A gene and the milk yield of 192 Anatolian buffalo raised in the Afyonkarahisar region. The total of 13 polymorphisms were identified through Sanger sequencing, including five in the 7th intron, seven in the 8th intron, and only one of them in the 8th exon. The relationship between adjusted milk yields and SNPs was analyzed using ANOVA (one-way analysis of variance). The association between milk and SNP 12237 (g+12237 C>T) was located in exon 8 of the STAT5A gene. Therefore, milk yield was found to be significant ($P<0.01$). Buffaloes with the CC genotype were found to produce less milk than buffaloes with the CT genotype for this SNP. In conclusion, our findings suggest that SNP 12237 can be used in buffalo breeding programs to improve milk yield.

Keywords: Anatolian buffalo, Milk yield, SNP, STAT5A

✉**Corresponding author:** emsal.ibis@ksbu.edu.tr

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Introduction

The water buffalo *(Bubalus bubalis)* is a member of the Bovidae family and is economically significant, distributed across South Asia, the Middle East, South America, Southern Europe and North Africa. The Mediterranean buffalo, which originated from the Asian water buffalo, accounts for approximately 3% of the global river buffalo population (18, 23). The Anatolian buffalo, a native breed of Türkiye, is classified as a Mediterranean-type river buffalo. It is characterized by a black or grey coat with sparse hair (6, 7, 14). River buffalo are valued for their ability to produce high-quality milk and meat even when fed low-quality feed. Compared with cow's milk, buffalo milk has lower levels of cholesterol, sodium, and potassium but higher levels of calcium, phosphorus, and certain vitamins (10, 23). The STAT5A gene plays a significant role in various physiological processes, including embryo viability, milk production, and growth traits in cattle (15, 19). The STAT5A gene in the Bubalus bubalis is 21,077 base pairs in length and consists of 19 exons, encoding a total of 794 amino acid chains (17). In Türkiye, the primary objective of buffalo husbandry is the production of milk and meat. Furthermore, buffalo are preferred due to their resistance to many infectious diseases, ability to adapt to harsh conditions, low rearing costs, and suitability for low-income farmers (20). Mammary gland tissues undergo cycles of cell proliferation, differentiation, and apoptosis during adulthood. These processes are regulated by different hormones, growth factors, and coding genes. These processes involve multiple signaling pathways with distinct regulatory functions. Among these, the JAK/STAT signaling pathway has been identified as a critical factor in the development of the mammary gland and the synthesis of milk proteins (11). It has also been established as the primary mediator of growth hormone (16). The STAT5A gene has been demonstrated to play a significant role in various physiological processes,

including embryo viability, milk production, and growth traits in cattle (15, 19). In dairy animals such as buffalo, the STAT5A gene has been demonstrated to regulate the expression of genes associated with milk yield, thereby playing a pivotal role in the process of milk production. However, studies related to this gene in buffalo are limited. It is anticipated that this study will make a significant contribution to the extant literature on buffalo husbandry. The objective of this study was to detect polymorphisms in the STAT5A gene in Anatolian water buffalo, investigate their relationship with milk yield, and contribute to the breeding efforts.

Materials and Methods

A total of 192 hair samples were collected from Anatolian water buffalo raised in the Afyonkarahisar province. These samples were used for the extraction of DNA. These samples were studied as a part of a project entitled "Development of a Genetic Diagnostic Kit for Breeding Selection in Terms of Growth and Milk Yield Traits in Anatolian Buffalo," and supported by the General Directorate of Agricultural Research and Policies (TAGEM), Ministry of Agriculture of the Republic of Türkiye. In this study, monthly milk yield from buffalo heifers was recorded and used throughout the lactation period from 2012 to 2019.

DNA isolation was performed utilizing a modified version of the Boom et al. method (3), which was adapted for use with 500 nm magnetic beads. The purities of the DNA samples were determined at 260/280 and 260/230 wavelengths using a Multiskan GO instrument (ThermoFisher Sci.), while the quantity of DNA was measured using Qubit 2.0 (Invitrogen).

PCR Analysis: The primers were designed by the FastPCR Professional 6.1.2 package program (13) based on the reference STAT5A gene sequence (NW_005784710.1) obtained from the NCBI database. The program was also utilized to assess potential dimer formation among the primers. The primer sequences, including the Tm (Temperature melting) degrees, and the base pairs, are presented in Table 1. In summary, the 25-µL PCR mixture consists of 2 µL of DNA (30–40 ng/µL), 10 pmol of each primer, 2 mM $MgCl_2$, 1× PCR buffer, 0.5 µL dNTP mix, 1 U Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA), and 17.9 µL of double-distilled H_2O. The PCR reaction was carried out for 2 min at 94°C (pre-denaturation), 45 s at 94°C (denaturation), 30 s at 58 or 62°C (annealing), and 1 min at 72°C (extension) for 35 cycles. The final extension was at 72°C for 10 min. PCR products were purified using Exonuclease I (EN0582; Thermo, Vilnius, Lithuania) and FastAP Thermosensitive Alkaline Phosphatase (EF0651; Thermo).

Sequencing Analysis: Subsequently, the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, 4337455) was utilized to perform the sequencing PCR, in accordance with the manufacturer's protocol. The amplicons were then purified using a BigDye XTerminator purification kit (4376487; Thermo), and the base sequences were determined using an Applied Biosystem 3500 Genetic Analyzer (Thermo Fischer).

Finally, the process of nucleotide sequencing was conducted using the Sequencher 5.6.1 software (Gene Codes Corporation, Ann Arbor, MI). The consensus sequences were subsequently aligned using the BioEdit software (9), and single-nucleotide polymorphisms (SNPs) were identified.

Statistical Analysis: The genotype frequency, total allele count, allele frequencies (Xi), and heterozygosity values (He) of SNPs were calculated using the Genetics package (22), developed in R. The Haploview program (2) was utilized to generate haplogroups. Only those haplogroups exhibiting the lowest allele frequency and a Hardy-Weinberg P value greater than 0.001 were incorporated into the analysis. The four-gamete rule was considered during the block creation process. The associations between SNPs and milk yield in a lactation period were analyzed using ANOVA in the Minitab software. Only the first lactation records of buffalo heifers were used in the association analysis. The lactation milk yields were adjusted for a number of variables, including village, calving year, calving season, calving age, and lactation length according to the following model;

$$Y = \mu + V + CY + CS + CA + LL + e,$$

Where, Y is the lactation milk yield, µ = overall mean, V = effect of village, CY = effect of calving year, CS = effect of calving season, CA = effect of calving age, LL= effect of lactation length and e = random error N (0, σ^2) related to each observation (8). These adjusted milk yield records were then subjected to analysis to assess the effects of SNPs.

Table 1. Primers used in the study (STAT5A *Bubalus Bubalis* Gene ID:102415442)

Forward / Reverse	Primers (5'→3')	Tm (°C)	Base length
F1 (F11159)	TCCTCCAGCTCAGTTTGCCCA	58 °C	1003 bp
R1 (R12162)	TCTGCCAGATGATCTCCGCCA		
F2 (F11950)	TTGGAAGGCAGGGCATCTCTGC	62 °C	929 bp
R2 (R12879)	CAGCGTACTTGCGGGTGTTCTC		

Table 2. Genotype and allele frequencies, heterozygosity and Hardy-Weinberg values in the STAT5A gene

SNP	Region	Allele	Allele frequencies	Genotypes	Genotype frequencies	Heterozygosity values	Hardy-Weinberg chi-square	Hardy-Weinberg P value
11865	Intron 7	C	0.73	CC	0.46	0.395	24.704	$2.36x10^{-8}$
		T	0.27	CT	0.54			
11909	Intron 7	G	0.74	GG	0.50	0.381	14.825	$6.09x10^{-5}$
		A	0.26	GA	0.49			
				AA	0.01			
11939	Intron 7	G	0.25	AA	0.50	0.376	20.222	$4.72x10^{-7}$
		A	0.75	GA	0.50			
12020	Intron 7	C	0.15	AA	0.70	0.257	5.766	0.0161
		A	0.85	AC	0.30			
12098	Intron 7	C	0.20	TT	0.60	0.322	11.453	0.0002
		T	0.80	TC	0.40			
12237	Ekzon 8	C	0.82	CC	0.63	0.301	9.262	0.0011
		T	0.18	CT	0.37			
12318	Intron 8	G	0.95	GG	0.90	0.099	0.552	1.0000
		A	0.05	GA	0.10			
12323	Intron 8	A	0.95	AA	0.90	0.094	0.493	1.0000
		G	0.05	AG	0.10			
12372	Intron 8	G	0.80	GG	0.60	0.318	11.065	0.0003
		A	0.20	GA	0.40			
12446	Intron 8	G	0.74	GG	0.51	0.386	6.044	0.0203
		T	0.26	GT	0.46			
				TT	0.03			
12464	Intron 8	G	0.95	GG	0.90	0.094	0.496	1.0000
		C	0.05	GC	0.10			
12598	Intron 8	G	0.78	GG	0.57	0.341	13.984	$3.58x10^{-5}$
		A	0.22	GA	0.43			
12646	Intron 8	C	0.87	CC	0.73	0.233	4.404	0.0474
		T	0.13	CT	0.27			

Results

In this study, SNPs were identified in the 1720 bp region between the sixth and ninth exons of the STAT5A gene in a total of 192 buffalo samples. A total of 13 polymorphisms were identified, including five in the seventh intron, seven in the eighth intron, and one in the eighth exon. The values for the allele and genotype frequencies, the heterozygosity (He), and the Hardy-Weinberg values are shown in Table 2.

In the haploview analysis conducted to determine the linkage disequilibrium. The relationships between SNPs in the examined region are illustrated in Figure 1. The figure reveals that the SNPs form two distinct blocks: the first block consists of SNPs 12446, 12464, and 12646, while the second block includes SNPs 12237, 12318, 12323, and 12372. SNPs 11865, 11909, 11939, and 12598 were excluded from the analysis due to the deviation from Hardy-Weinberg equilibrium ($P<0.0001$). In the first block, the haplotypes CGG, CGT, TGG, and CCG were identified. Among these, CGG (0.555) and CGT (0.261) appeared to be the most prevalent. In the second block, the GAGC, AAGC, GAGT, and GGAC haplotypes were identified. The haplotypes GAGC (0.577) and AAGC (0.189) appeared to be most prevalent. Furthermore, an increased prevalence was observed inanimals carrying the CGGGAGC, CGTGAGC, and CCGAAGC haplogroups.

ANOVA was performed to determine the relationships between SNPs and milk yield averages obtained from the database program called "Buffalo Star" (21), which are adjusted to various environmental factors. The genotypes, animal numbers and average milk yields obtained as a result of this analysis are given in Table 3.

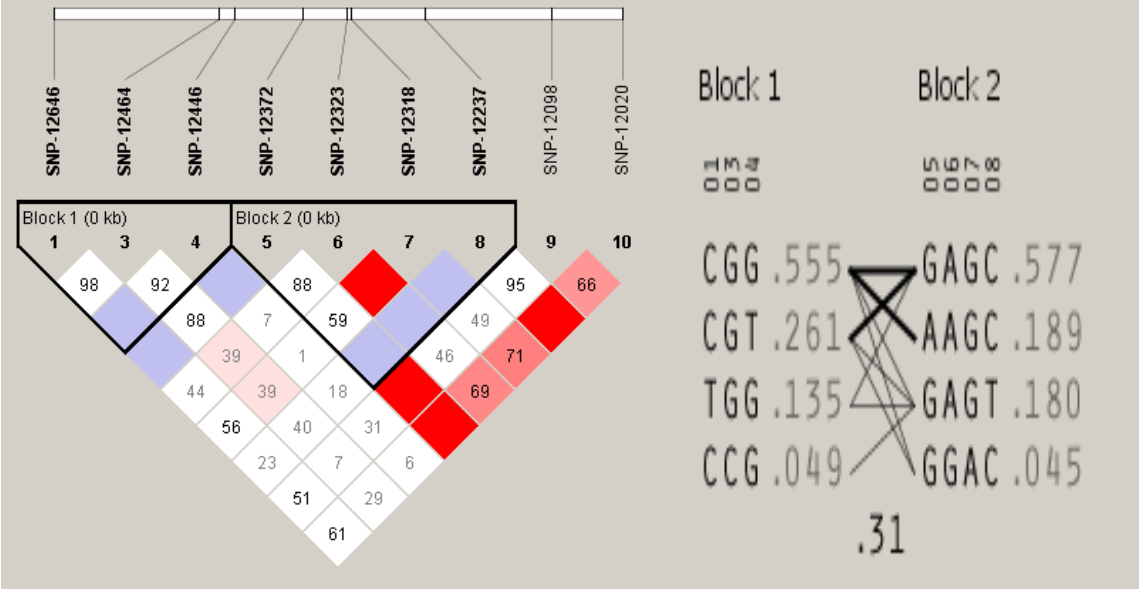

Figure 1. Haploview image of SNPs identified in the STAT5A gene

Table 3. Variance analysis table showing the relationships between genotypes and corrected milk yield averages in Anatolian buffalo.

SNP	Region	Genotypes	N	Adjusted milk yield average	P value
11865	Intron 7	CC	80	893.5	0.767
		CT	91	884.6	
11909	Intron 7	GG	85	892.3	0.970
		GA	84	885.0	
		AA	2	897.0	
11939	Intron 7	AA	86	890.0	0.933
		GA	85	887.5	
12020	Intron 7	AA	119	893.4	0.645
		AC	52	878.2	
12098	Intron 7	TT	101	885.5	0.796
		TC	70	893.4	
12237	Exon 8	CC	109	856.8	0.005**
		CT	62	944.9	
12318	Intron 8	GG	152	895.3	0.220
		GA	19	836.5	
12323	Intron 8	AA	153	894.2	0.293
		GA	18	842.6	
12372	Intron 8	GG	106	888.7	0.997
		GA	65	888.8	
12446	Intron 8	GG	86	882.2	0.446
		GT	79	902.4	
		TT	6	802.8	
12464	Intron 8	GG	155	884.4	0.363
		GC	16	931.4	
12598	Intron 8	GG	99	885.1	0.778
		GA	72	893.7	
12646	Intron 8	CC	124	881.4	0.428
		CT	47	908.1	

** (P<0.01 high level of statistical significance)

The statistical analysis revealed that there was not a statistically significant relationship between the milk yields and the SNPs in the seventh as well as in the eighth introns. However, a significant association was observed in the milk yields located in the SNP 12237 (g.12237C>T), in the eighth exon of the STAT5A gene ($P<0.01$). Buffaloes exhibiting the CC genotype at this SNP were found to produce less milk compared to those with the CT genotype. The analyses did not reveal any buffalo with the TT genotype.

Discussion and Conclusion

The geographical conditions of Türkiye provide a suitable environment for the husbandry of Anatolian buffalo. It is hypothesized that the implementation of breeding studies and the utilization of genetic markers breeding process will contribute to the economic development of the country by enhancing milk yield in buffalo. Among these genetic markers, STAT5A plays a significant role in induces an increase in milk, and it regulates the expression of genes for milk proteins. STAT genes are responsible for encoding intracellular transcription factors. These factors regulate gene expression by binding to the specific DNA sequences within the nucleus. Research has identified the role of STAT5A in cattle, goats and sheep and has been associated with milk fatty acid profiles and milk yield (1, 4). This study sought to determine the polymorphisms in the STAT5A gene of Anatolian buffalo, and a total of 13 SNPs were identified. Among these SNPs, five were located in intron 7, one in exon 8, and seven in intron 8. Despite the fact that the SNPs were located within the same gene, two distinct blocks were identified during the Haploview analysis. This finding suggests that the crossing over may occur in this region. In addition, the relationship analysis between milk yield and SNPs showed that there is a significant correlation between milk yield and SNP 12237, located in exon 8. The present study also showed that the mean milk yield of buffalo possessing the CT genotype in this SNP exceeded that of buffalo with the CC genotype.

Kale and Yadav (12) reported that there was a significant effect of the SNP detected in exon 7 on 305-day milk yield in their study on STAT5A gene polymorphisms in Murrah buffalo. Selvaggi et al. (19) investigated the association between STAT5A gene polymorphism and milk composition in Agerolese-breed cattle, finding that cows with the TT genotype produced milk with higher fat and protein content compared to those with the TC genotype. Coizet et al. (5) investigated the association between STAT5A gene polymorphisms and milk yield in 491 Italian buffalo cows, identifying three SNPs in the intronic region and one in the exonic region. The results of the study indicated that in STAT5A_c989+344, located in intron 8, animals with the TT genotype exhibited a higher milk protein percentage compared to those with the TC and CC genotypes. The findings of this study are consistent with those reported by Coizet et al. (5). İbiş and Erdoğan (10) identified 10 SNPs, including one in intron 6, five in intron 7, three in intron 8, and one in exon 8 of the STAT5A gene in their study on 96 Anatolian buffalo cows. In this study, a comparable number of SNPs were identified in intron 7 and exon 8 of Anatolian buffalo cows. However, four additional SNPs were detected in intron 8. Naveed et al. (16) identified a total of nine single-nucleotide polymorphisms (SNPs) in the STAT5A gene in their study on Nili Ravi buffalo in Pakistan, six of which were located in the intron and three in the exon. The researchers reported that serine (AGT) was converted to asparagine (AAT) due to the G→A base change in the 5th exon; however, the relationship between this conversion and milk yield was not investigated.

In conclusion, research on various animal species has demonstrated that the STAT5A gene exerts a significant influence on the development of the mammary gland, the release of prolactin as well as the quantity of milk produced. The STAT5A gene plays a crucial role in the development of the mammary gland, including synthesizing milk proteins, regulating mammary gland cell proliferation, mediating prolactin (PRL) and growth hormone (GH) signaling, fertilization, and embryonic development. Therefore, a more exhaustive investigation of this gene in buffalo, in conjunction with the association of identified single-nucleotide polymorphisms (SNPs) with production traits and their incorporation into breeding programs, could substantially contribute to the advancement of Türkiye buffalo breeding.

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Ethical Statement

This study was approved by the Afyon Kocatepe University Animal Experiments Local Ethics Committee with the decision numbered 49533702/155 dated 16.10.2019.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

EI and ME conceived the idea, planned the manuscript, provided scientific support and contributed to the interpretation of the results.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

References

1. **An XP, Hou JX, Zhao HB, et al** (2013): *Polymorphism identification in goat DGAT1 and STAT5A genes and association with milk production traits.* Czech J Anim Sci, **58**, 321-327.
2. **Barrett JC, Fry B, Maller J, et al** (2015): *Haploview: analysis and visualization of LD and haplotype maps.* Bioinformatics, **21**, 263-265.
3. **Boom R, Sol CJA, Sal Imans MMM, et al** (1990): *Rapid and simple method for purification of nucleic acids.* J Clin Microbiol, **28**, 495-503.
4. **Brym P, Kaminski S, Rusc A** (2004): *New SSCP polymorphism within bovine STAT5A gene and its associations with milk performance traits in Black-and-White and Jersey cattle.* J Appl Genet, **45**, 445-452.
5. **Coizet B, Frattini S, Nicoloso L, et al** (2018): *Polymorphism of the STAT5A, MTNR1A and TNFa genes and their effect on dairy production in Bubalus bubalis.* Ital J Anim Sci, **17**, 31-37.
6. **Çelikeloğlu K, Koçak S, Erdoğan M, et al** (2019): *The investigation of viability and body measurements for water buffalo calves.* Turk J Vet Anim Sci, **43**, 60-67.
7. **Çınar MU, Özsoy T, Beyzi SB, et al** (2019): *Milk and fatty acid composition of Anatolian water buffalo (Bubalus bubalis) from different provinces.* Buffalo Bull, **38**, 107-118.
8. **Erdoğan M, Tekerli M, Çelikeloğlu K, et al** (2021): *Associations of SNPs in GHR gene with growth and milk yield of Anatolian buffaloes.* Turk J Vet Anim Sci, **45**, 1080-1086
9. **Hall TA** (1999): *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.* Nucl Acids Symp Ser, **41**, 95-98.
10. **İbiş E, Erdoğan M.** (2019): *The determination of polymorphisms between 6-9 exons of the water buffalo STAT5A gene.* Eurasian J Vet Sci, **35**, 230-235.
11. **Jiao BL, Zhang XL, Wang SH, et al** (2019): *MicroRNA-221 regulates proliferation of bovine mammary gland epithelial cells by targeting the STAT5a and IRS1 genes.* J Dairy Sci, **102**, 426-435
12. **Kale DS, Yadav BR** (2012): *PCR-SSCP within STAT5A gene and its association with milk yield in Murrah buffalo.* Indian Vet J, **89**, 16-20.
13. **Kalendar R, Lee D, Schulman AH** (2009): *FastPCR software for PCR primer and probe design and repeat search.* Genes Genomes Genomics, **3**, 1-14.
14. **Koçak S, Tekerli M, Çelikeloğlu K, et al** (2019): *An investigation on yield and composition of milk, calving interval and repeatabilities in riverine buffaloes of Anatolia.* JAPS, **29**, 650-656.
15. **Li S, Huang S, Qiao S, et al** (2018): *Cloning and functional characterization of STAT5a and STAT5b genes in buffalo mammary epithelial cells.* Anim Biotechnol, **31**, 59-66.
16. **Naveed M, Nadeem A, Javed M, et al** (2021): *Evolutionary divergence of Signal Transducer and Activator of Transcription 5A (STAT5A) gene in riverine buffalo.* Pakistan J Zool, **53**, 1459-1465.
17. **Ouerghi S, Jemmali B, Trab I, et al** (2017): *Identification of polymorphism at the STAT5 locus in dairy cows in Tunisia.* J New Sci, **1**, 11-14.
18. **Öz S, Alkoyak K, Küçükersan S** (2022): *Effects of calving year, season, and age on some lactation traits of Anatolian buffaloes reared at farmer conditions in Turkey.* Ankara Univ Vet Fak Derg, **69**, 157-162.
19. **Selvaggi M, Albarella S, Dario C, et al** (2017): *Association of STAT5A gene variants with milk production traits in Agerolese cattle.* Biochem Genet, **55**, 158-167.
20. **Soysal Mİ, Genç S, Aksel M, et al** (2018): *Effect of environmental factors on lactation milk yield, lactation length and calving interval of Anatolian buffalo in Istanbul.* JASP, **1** ,93-97
21. **Tekerli M** (2019): *Manda Yıldızı: Buffalo Recording Software.* Ver. 2019, 5.00, 2015-2019. Afyon Kocatepe Üniversitesi. Afyonkarahisar, Türkiye.
22. **Warnes GR** (2003): *The genetics package.* R News, **3**, 9-13.
23. **Zhang Y, Colli L, Barker JSF** (2020): *Asian water buffalo: Domestication, history and genetics.* Anim Genet, **51**, 177-191.