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Identification of novel single nucleotide polymorphisms in the growth hormone (*GH*) gene in Anatolian water buffalo (*Bubalus bubalis*) populations in Turkey

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ABSTRACT - This study was conducted to investigate the growth hormone (*GH*; *somatotropin-like)* gene polymorphisms in 150 water buffalo (*Bubalus bubalis*) from different regions of Turkey. 404 bp long partial intron 4, exon 5, 3' UTR regions of the *GH* gene (also called *GH/Alu*I locus) and 347 bp long exon-intron 3 and partial exon 4 regions of the *GH* gene (also called *GH/Msp*I locus) were amplified, and their PCR products analyzed via DNA sequencing method. Seven genotypes due to twenty single nucleotide polymorphisms (SNP) and one deletion/insertion were identified in a 347 bp long region of the *GH/Msp*I locus. A missense mutation from glycine to glutamate amino acid and four silent mutations in the serine, threonine, and asparagine amino acids were determined in the exon 3 region of the *GH* gene. Four genotypes due to eight SNP were identified in a 404 bp long region of the *GH/Alu*I locus. A missense mutation from lysine to arginine amino acid and six silent mutations in Leucine, aspartate, histidine, lysine, arginine, and cysteine amino acids were revealed in the exon 5 region of the *GH* gene. The partial DNA sequence of the *GH* gene in water buffalos was reported, and these sequences were deposited at the NCBI Genbank database with the accession numbers MN266903-MN266909 and MN530973-MN530976. These SNP may have an effect on economic (such as body composition) and carcass traits, reproduction, and milk yield and content in water buffalo populations and may prove to be useful for water buffalo breeding.

Keywords: biodiversity, breeding, DNA sequencing, growth traits

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1. Introduction

The water buffalo or domestic water buffalo originated in India, Southeast Asia, and China. The two classes of domestic water buffalo are swamp buffalo (*Bubalus carabanesis*) and river buffalo (*Bubalus bubalis*). While the river buffalo has 50 chromosomes, the swamp buffalo has 48 (Mishra et al., 2015). Water buffalo are genetically adapted to harsh environmental conditions such as low-quality pasture, parasites, and pathogens, so they can survive easily and are more productive (Andreas et al., 2010; Soysal, 2013). Anatolian water buffalo reared in Turkey generally have a dark coat color and originated from the Mediterranean subgroup of river buffalo (Soysal, 2013; Konca and Akyüz, 2017).

Water buffaloes have been reported to be of great importance to the lives of farmers and thus to the economies of many countries worldwide (Hussain et al., 2017). The number of water buffaloes in the world has increased rapidly over the past few decades, and according to FAO statistics, there are about 201 million buffaloes in the world (FAO, 2017). Most of world buffaloes are found in Asia (%97), Egypt, and southern and south-eastern Europe. Besides, buffaloes have played an important role in the rural

economy of developing Asian country from ancient times. India has the highest water buffalo population with around 113 million animals. Pakistan is the second country that has around 37.7 million animals. The third country is China with around 23.4 million animals.

According to 1974 FAO statistics, at that time there were one million buffalo head in Turkey. From 1984 to 1998, there has been a decrease in the buffalo breeding population of 75%, and the reason for this decrease in water buffaloes is the preference for cattle in the Aegean and Marmara regions, where many buffaloes were found. In this period, all the improvement efforts for genotypes were only practiced in cattle in Turkey. Thanks to the incentives introduced for water buffalo husbandry in recent years, the water buffalo population has risen to 180.826 head (HAYGEM, 2019). Since 2013, the National Anatolian Water Buffalo Breeding Project has been implemented in Turkey by the Republic of Turkey Ministry of Agriculture and Forestry with the cooperation of different universities, research institutes, and water buffalo breeder associations under the condition of breeders. The number of water buffaloes in Turkey has increased from 84,000 to 142,000 over the last ten years (FAO, 2019).

Water buffalo husbandry in Turkey is performed in some provinces of the Black Sea, Marmara, and Central Anatolian regions. The provinces with the highest amount of water buffalo existence are listed as Samsun, Istanbul, Diyarbakır, Tokat, Bitlis, Muş, Afyon, Kayseri, Sivas, and Amasya (Ermetin, 2017). They are not only draught animals, but also a source of meat, horns, skin, and particularly milk, which may be converted into cream, butter, yoghurt, and many kinds of cheese. In Turkey, these animals are mainly used for milk and meat production in these areas. The lactation milk yield and lactation length in Anatolian water buffaloes are between 800 and 1100 kg and about 180-280 days, respectively. It is demonstrated that they vary according to effects of environmental factors, care, and feeding. Especially in recent years, genetic improvement studies have started to increase milk yields in Turkey.

To identify high-quality genetic material for animal improvement programs, many researchers have used molecular genetics techniques to identify genes responsible for phenotypic variation associated with traits of economic interest. Methods have been developed for the selection of superior genotypes (Venturini et al., 2014). However, even with the development of molecular genetic methods to study genomic associations between various animals, such as cattle (Meredith et al., 2012), sheep (Di Gerlando et al., 2019), goats (Mucha et al., 2018), poultry (Xie et al., 2012), and pigs (Schneider et al., 2012), molecular information and tools for water buffaloes is limited. Genomic techniques are particularly attractive for animal improvement because of the ability to directly use DNA information for selection, allowing higher selective efficiency, a faster rate of obtaining genetic gains, and low cost compared with traditional selection based on phenotypic data (Schaeffer, 2006). Among the available genomic tools, the use of single-nucleotide polymorphism (SNP) markers is particularly effective for selecting traits measured in a single sex, such as milk yield and milk composition (Venturini et al., 2014). Several studies have examined buffaloes to identify SNP markers associated with milk components (Gil et al., 2013; Ahmadzadeh et al., 2019; Liu et al., 2018). One of the most important of this SNP marker is the growth hormone (*GH*) gene region.

The *GH* is an anabolic hormone that is synthesized in the anterior pituitary (Ayuk and Sheppard, 2006). It plays a crucial role in physiological regulation, affecting growth, body composition and carcass traits, reproduction, proliferation of mammary cells, and lactogenesis (Yardibi et al., 2009; Kour et al., 2018; Amiri et al., 2018). Due to these crucial biological functions, *GH* is considered a useful candidate gene for increasing milk and meat yield in buffalo via marker-assisted selection (MAS) programs (Othman et al., 2012). The *GH* is a member of the somatolactogenic hormone family, which includes prolactin, placental lactogen, and antlers hematopoietic growth factors (Cosman et al., 1990).

The GH gene is very conservative among the bovine, goat, sheep species, while the water buffalo *GH* gene has 94% sequence identities in the DNA sequence (NCBI, 2019a). The *GH* gene is localized on chromosome 11 in sheep and on chromosome 19 in goats, cattle, and water buffaloes. While the *GH* gene contains five exon counts in water buffalo, sheep, cattle, and goats, the water buffalo *GH* gene consists of five exons, 217 amino acid residues, and are 1854 bp long (NCBI, 2019b). The GH amino acid residues are 99% identifiable among water buffaloes (AGG09123), bovine (ABY61238), goats (ABC18164), red deer (CAJ18232), and 98% in sheep (ADM67559) (NCBI, 2019c).

Three novel polymorphisms have been identified in exon 5 of the *GH* gene in bovine (Yao et al., 1996). Many studies have been conducted regarding the relationship between *GH* gene polymorphisms and traits in livestock (Yardibi et al., 2009; Öner et al., 2011; Korkmaz Ağaoğlu and Akyüz, 2013; Akyüz et al., 2013; Akçay et al., 2015; Amiri et al., 2018; Kour et al., 2018; Ozdemir et al., 2018), but limited research was found concerning water buffalo (Andreas et al., 2010; Shi et al., 2012; Othman et al., 2012; Hussain et al., 2014). Andreas et al. (2010) identified variation in *GH/Alu*I locus in Indonesian buffalo. Shi et al. (2012) revealed genetic variation in *GH/Alu*I and *GH/Msp*I loci in Murrah river buffalo, Nili-ravi river buffalo, Chinese swamp buffalo, and Murrah-nili-swamp crossbreed buffalo. Hussain et al. (2014) investigated the *GH/Alu*I locus in the local buffalo of Iraq and Mesopotamian buffalo (Hussain, 2015) using the PCR-RFLP technique. Similarly, Konca and Akyüz (2017) analyzed the same locus in the *GH* gene of Anatolian water buffalo. The *GH/AluI* locus was identified by researchers, but no study that identifies the *GH* gene DNA sequence variation could be found for indigenous Anatolian buffalo. Thus, the purpose of the present study was to investigate the *GH* gene polymorphisms in Anatolian water buffalo using the DNA sequencing method.

2. Material and Methods

All procedures involving manipulation of the animals were conducted in accordance with Turkish guidelines on animal welfare. This research was approved by the Ethics Committee on the Use of Animals, Republic of Turkey Ministry of Agriculture and Forestry, Institute of Pendik Veterinary Control Ethics Committee (case no. 2013/12).

In this study, the 150 unrelated Anatolian water buffalos blood samples used (0-6 months, 1-3 years; males, n = 46; females, n = 104) were collected from the East Anatolia (Bitlis, Diyarbakır, Muş, n = 40), Black Sea (Amasya-Merzifon, Samsun, Tokat, Çorum, Giresun, Sinop, n = 50), Central Anatolia (Kayseri, Sivas, n = 17), and Aegean-Marmara (Afyon, Istanbul-Nakkaş/Danamandıra, Balıkesir, Bursa, Tekirdağ-Saray, n = 43) regions of Turkey (Figure 1). Blood samples were taken in 10 mL vacuum tubes, including EDTA as anticoagulant and stored at −20 °C until the DNA extraction. Genomic DNA was isolated using the phenol-chloroform extraction method.

The primer sequences for the 347 bp long region of the *GH* gene are F: 5'- GGACAGAGATACTCCATCCAG -3' and R: 5'- AGATGCGAAGCAGCTCCAAGT -3' (Shi et al., 2012). The primer sequences for the 404 bp long region of *GH* gene are F: 5'- TAGGGGAGGGTGGAAAATGGA -3' and R: 5'- GACACCTACTCAGACAATGCG -3' (Shi et al., 2012). For amplification reactions, the 20 µL PCR volume contained 50 ng genomic DNA, 2.5 µM of each primer, 1× PCR Buffer ($(NH_4)_2SO_4$), 200 µM dNTP, 2.0 mM $MgCl_2$, and 0.5 U of Taq DNA polymerase (Taq DNA Polymerase, Thermo Scientific, US) for two regions of *GH* gene. The cycling protocol was 5 min at 94 °C for the initial denaturation, 35 cycles of amplification; 94 °C for 30 s, 58.1 °C annealing for 45 s,

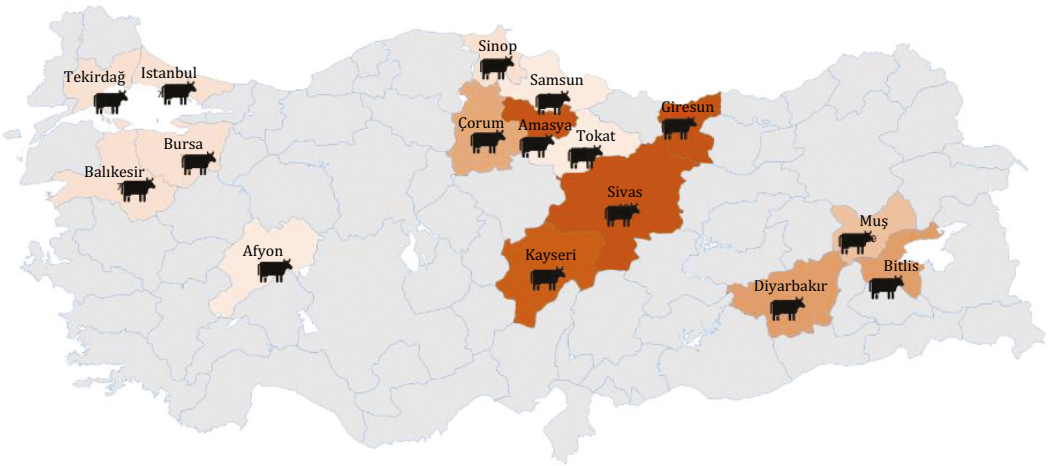

Figure 1 - Location of the Anatolian water buffalo samples collected in this study.

72 °C for 45s, and 15 min at 72 °C for the final extension of the two regions of the *GH* gene. Afterwards, the PCR products were checked on 2% agarose gel using horizontal electrophoresis, and the gels were stained using RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Inc., Korea).

The 347 bp and 404 bp regions of the *GH* gene were sequenced on an Applied Biosystems 3500XL Genetic Analyzer System (Applied Biosystems, USA) to identify the *GH* gene sequence. The PCR containing target SNP region was performed with suitable primers, and then Exosap was used to hydrolyze excess primers and nucleotides in the reaction. After that, sequencing reaction was performed by using BigDye Terminator v.3.1 Cycle Sequencing Kit (Thermo, USA). Amplicons were purified and efficiently sequenced by capillary electrophoresis. Sanger sequencing, which is a good option for SNP analysis, provided conclusive allele identification data. The sequences were analyzed and compared with the reference data to determine the target SNP region. The SNP region were found on the sequence for all samples and labelled. The single nucleotides were compared with the reference genome. If the nucleotide on SNP region is different from reference nucleotide, we called it "mutant allele" for sample, while we called "wildtype allele" if the nucleotide was the same with reference nucleotide. Also, for the heterozygote allele, two peaks were observed from sequence data on SNP region. The sequences were aligned with ChromasPro 2.4.3 program (Technelysium Pty Ltd) and BioEdit v7.0.9 Sequence Alignment Editor with Clustal W multiple alignment modules (Hall, 2011).

3. Results

In our study, the 347 bp of the *GH* region spanning the exon-intron 3, partial exon 4 regions of the *GH* gene and the 404 bp of the *GH* region spanning the partial intron 4, exon 5, 3' UTR regions of the *GH* gene in water buffalo include 39 and 67 amino acids, respectively. A total of 217 amino acids in water buffalo and their comparison with the GH amino acid residues of different species and the new amino acid changes found in this study are shown in Table 1. The four silent mutations in serine (TCC>TCT),

Table 1 - Comparison of GH amino acid residues with different species

Species		GH amino acid residue	
Buffalo	1	MMAAGPRTSLLLAFALLCLPWTQVVGAFPAMSLSSLFANAVLRAQHLHQLAADTFKEFER	60
Cattle	1	----------G----------	60
Goat	1	----------G----------	60
Sheep	1	----------TM----------G----------	60
Red Deer	1	----------E----------G----------	60
Buffalo	61	TYIPEGQRYSIQNTQVAFCFSETIPAPTGKNEAQQKSDLELLRISLLLIQSWLGPLQFLS	120
Cattle	61	----------	120
Goat	61	----------G	120
Sheep	61	----------	120
Red Deer	61	----------	120
MN266903	61	--------E----------	120
Buffalo	121	RVFTNSLVFGTSDRVYEKLKDLEEGILALMRELEDGTPRAGQILKQTYDKFDTNMRSDDA	180
Cattle	121	----------V----------	180
Goat	121	----------	180
Sheep	121	----------V----------	180
Red Deer	121	----------	180
MN530974	121	----------R----------	180
Buffalo	181	LLKNYGLLSCFRKDLHKTETYLRVMKCRRFGEASCAF	217
Cattle	181	----------	217
Goat	181	----------	217
Sheep	181	----------	217
Red Deer	181	----------	217

threonine (ACA>ACG), asparagine (AAC>AAT), and serine (TCG>TCA) amino acids and a missense mutation from glycine to glutamate (GGA>GAA) amino acid were determined in the exon 3 region of the *GH* gene. Four genotypes due to eight SNP were identified in the 404 bp long region of the *GH* gene. The six silent mutations in leucine (CTG>CWG), aspartate (GAC>GAT), histidine (CAC>CAT), lysine (AAA>AAG), arginine (CGG>AGG), and cysteine (TGC>TGT) amino acids and a missense mutation from lysine to arginine (AAG>AGG) amino acid were revealed in the exon 5 region of the *GH* gene. This sequence was identified, and the partial DNA sequence of the *GH* gene in water buffalo was reported for the first time in this study; these sequences were deposited in the NCBI Genbank database with the access numbers MN266903-MN266909. The nucleotide changes and frequency of the studied *GH* gene regions are shown in Table 2. Nucleotide mutations observed in a small number of individuals were controlled by sequencing for the second time. The nucleotide changes can be observed in Figure 2 at

Table 2 - Variation of nucleotide identified in the *MspI* and *Alu*I regions from *GH* gene on water buffalo in Turkey

Locus	Position	Nucleotide change				Genbank accession number[4]	Type of mutation
		Bos taurus[1]	Buffalo[2]	AWT	AWB[3]		
	2108	C	C	Y(C/T)	Y (0.006)	MN530973	Transition
	2139-42	AGCT	AGCT	-	D (1.000)	All Sequences	Deletion
	2142	T	T	W(T/A)	W (0.006)	MN530974	Transversion
	2149	T	T/C	T	T (0.013)	MN530975-6	Transition
	2178	A	A	R(A/G)	R (0.006)	MN530974	Transition
*GH/Alu*I	2272	T	C	T	T (0.013)	MN530975-6	Transition
	2275	G	A/G	G	G (0.013)	MN530975-6	Transition
	2291	A	A/C	M(A/C)	M (0.006)	MN530976	Transition
	2329	T	C	T	T (0.013)	MN530975-6	Transition
	2356	T	-	-	D (1.000)	All Sequences	Deletion
	2380	T	A	A/T	T (0.013)	MN530975-6	Transversion
	1427	T	C	C/T	T (0.013)	MN266903	Transition
	1448	G	A	A/G	G (0.013)	MN266905	Transition
	1457	T	C	C/T	T (0.020)	MN266905-7	Transition
	1475	A	G	A/G	A (0.013)	MN266905-7	Transition
	1485	A	A	A/C	C (0.006)	MN266905	Transition
	1490	C	C	C/A	A (0.006)	MN266905	Transition
	1520	C	C	C/T	T (0.006)	MN266905	Transition
	1541	-	T	T	T (1.000)	MN266903	Insertion
	1543	C	C	C/T	T (0.006)	MN266905	Transition
	1551	-	G	G	G (1.000)	MN266903	Insertion
	1559	C	C	C/T	T (0.006)	MN266905	Transition
	1560	G	A	A/G	G (0.013)	MN266907	Transition
	1571	C	C	C/T	T (0.006)	MN266905	Transition
*GH/Msp*I	1580	G	C	C/G/A	G (0.006) A (0.006)	MN266905 MN266907	Transition
	1584	G	A	A/G	G (0.013)	MN266907	Transition
	1585	T	A	A/T/G	T (0.006) G (0.006)	MN266905 MN266907	Transition
	1613	G	G	G/T	T (0.006)	MN266905	Transition
	1616	C	C	C/T	T (0.006)	MN266905	Transition
	1658	A	A	A/G	G (0.020)	MN266905	Transition
	1665	A	A	A/G	G (0.006)	MN266905	Transition
	1666	G	A	A	A (1.000)	MN266903	Transition
	1667	A	G	G	G (1.000)	MN266903	Transition
	1685	G	G	A	A (0.006)	MN266905	Transition
	1692	C	T	T	T (1.000)	MN266903	Transition

[1] GenBank accession number M57764 cattle.
[2] GenBank accession number AJ011533 water buffalo sequences used as a reference.
[3] Frequency of nucleotide changes.
[4] Genbank Access Numbers that were identified and deposited as a result of this study.

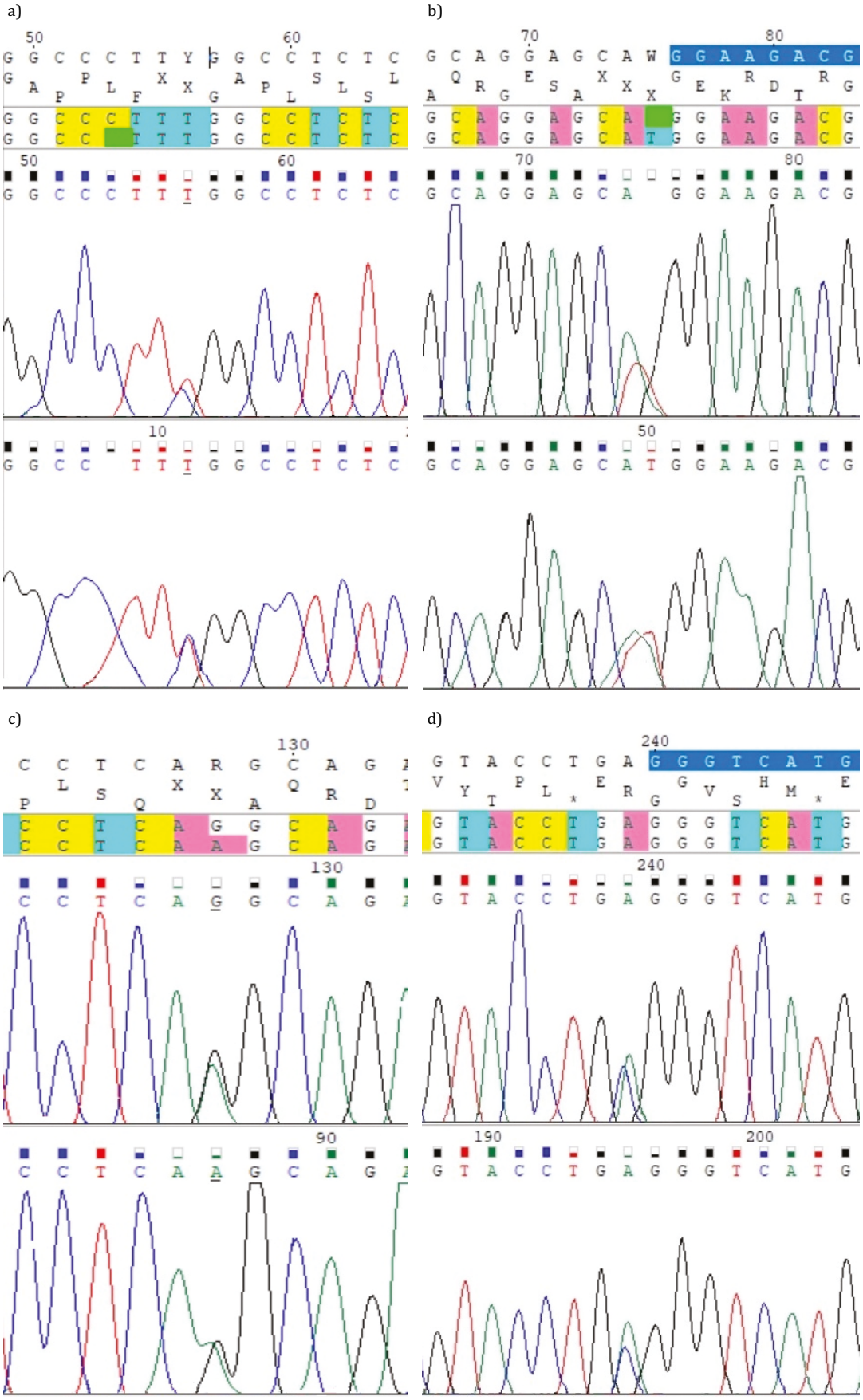

Figure 2 - Nucleotide changes seen in the studied samples of the *GH*/*Alu*I locus at positions a) 2108. (Y), b) 2142. (W), c) 2178. (R), and d) 2291. (M) of the cattle *GH* gene (M57764).

the studied samples of the *GH/Alu*I locus at positions 2108. (Y), 2142. (W), 2178. (R), 2291. (M) of the cattle *GH* gene (M57764).

All studied samples have an *Msp*I endonuclease restriction site (C'CGG) (Figure 3). Therefore, 347 bp long exon-intron 3 and partial exon 4 regions of the *GH* gene were found monomorphic for *GH*/*Msp*I locus. For *GH/Alu*I locus, 147 samples have *Alu*I endonuclease restriction site (AG'CT). Besides, three samples have T>A transition for 1540. nucleotide of buffalo *GH* sequence of NCBI GenBank (KC107770) (Figure 4). The transition of T>A caused an amino acid change from leucine (L) to glutamine (Q). Genotype and allele frequencies of *Msp*I and *Alu*I endonuclease restriction sites are shown in Table 3.

Figure 3 - Position of primers based on GenBank (Accession number M57764) and position of *Msp*I endonuclease restriction site.

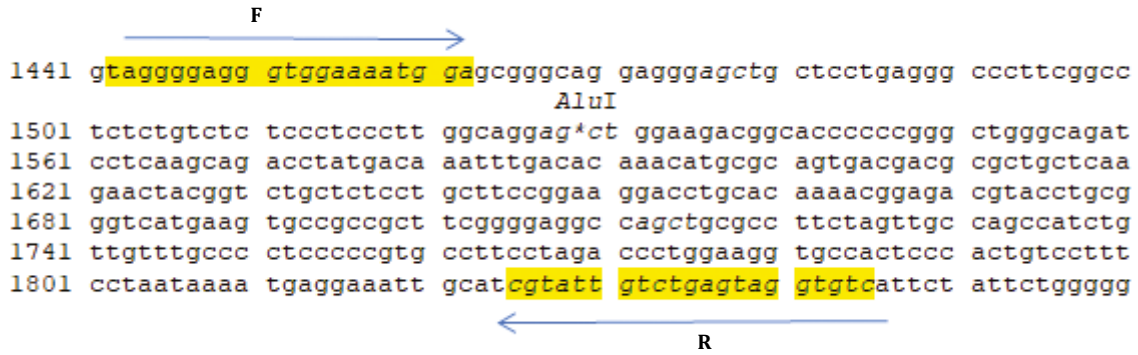

Figure 4 - Position of primers based on GenBank (Accession number KC107770) and position of *Alu*I endonuclease restriction site.

Table 3 - Genotype and allele frequencies of *Msp*I and *Alu*I endonuclease restriction sites

Locus		Anatolian water buffalo				
		Genotype frequency			Allele frequency	
		CC	CT	TT	C	T
*Msp*I	Observation	150.00	0.00	0.00		
	Expectation	150.00	0.00	0.00	1.00	0.00
	Frequency	1.00	0.00	0.00		
		LL	LQ	QQ	T	A
*Alu*I	Observation	147.00	3.00	0.00		
	Expectation	147.02	2.97	0.01	0.99	0.01
	Frequency	0.020	0.02	0.00		

4. Discussion

Growth hormone is a single chain protein secreted from the anterior lobe of the pituitary in all vertebrate species (Woychik et al., 1982; Gordon et al., 1983). This gene region has similar structural, biological, and immunological properties to the growth hormones of different species. The *GH* gene region polymorphism in cattle, sheep, and goats has been widely researched, but very few studies have been carried out on the *GH* gene variations in water buffalo populations (Shi et al., 2012; Hussain et al., 2014; Hussain, 2015), and these were generally conducted using the PCR-RFLP method. Therefore, in this study, *GH-MspI* and *GH-AluI* gene region polymorphisms (which have been determined to affect milk yield, fat, and protein ratios in cattle) were investigated for the first time using DNA sequencing for buffalo. The *GH* gene sequence is located between 15176933 and 15177335 bp at the NCBI GenBank database (Accession number NC_037547.1).

Andreas et al. (2010) investigated the *GH/Alu*I gene at 432 bp located on intron 4, exon 4-5, and found that the *GH/Alu*I locus is monomorphic and has LL genotype in five populations in Indonesia (Siborong-Borong-Medan, Lebak-Banten, Pandeglang-Banten, Semarang-Central Java, and Mataram-West Nusa Tenggara regions). Balogh et al. (2009) and Hussain et al. (2014) obtained results similar to Andreas et al. (2010). Konca and Akyüz (2017) amplified the 211 bp fragment for the *GH* gene exon 5 region. They found that LL genotype frequency was 0.755 and VV genotype frequency was 0.017 in the Anatolian water buffalo breed. Similar to Konca and Akyüz (2017), Hussain (2015) found that LL genotype frequency was 0.9409, LV genotype frequency was 0.0582, and VV genotype frequency was 0.0009. The studied samples have a different restriction pattern from Amiri et al. (2018), Konca and Akyüz, (2017), and Hussain (2015), which have amino acid change from leucine to glutamine. In our study, LL genotype frequency was 0.98; L allele frequency was found as 0.99. Nucleotide changes have been observed mostly in the east (Asian) part of Turkey, where the animals are raised in the border of the country. Breeding programs were not conducted for any traits in the individuals examined within the scope of this study.

Therefore, genetic variation is high in animals that were raised in the east (Asian) part of Turkey.

The *GH* induces cell growth with interaction of *GH* receptors binding with signal transducers and transcription activators. During the *GH* process, if *GH* receptor could not transmit the *GH* signal to IGF-1, a GH–GHR–IGF-1 axis dysfunction would have caused animal dwarfism. The mutations or amino acid change of GH or GHR could cause GHR dimerization, downstream signaling, resulting in defects in the number and diameter of muscle fibers (Carakushansky et al., 2003; Lin at al., 2018).

Shi et al. (2012) investigated the *GH-intron 3, GH-exon 5* with the SSCP method in Murrah river buffalo, Chinese swamp buffalo, Nili-ravi river buffalo, and Murrah-nili-swamp crossbreed buffalo. In *GH-Msp*I locus, five inter-specific SNP were found at the 48th, 181st, 201st, and 205th-206th sites between buffalo and dairy cattle. Also, according to Shi et al. (2012), two inter-specific SNP at the 83rd and 219th sites in GH-exon 5 were found between buffalo and dairy cattle.

Özşensoy and Kara (2019) investigated polymorphisms on exons 4 and 5 of the *GH* gene and on exon 10 of the GHR gene. They found that the meat yield trait since birth weight is related with the *GHR* genotypes in Anatolian water buffalo breed. Comparably with Özşensoy and Kara (2019), Ahmadzadeh et al. (2019) determined three genotypes in both the *GH* and *GHR* genes, and a statistically significant effect on body weight in Iranian water buffalo breeds. In Table 4, it can be observed the relationships between the *GH-Msp*I and *GH-Alu*I polymorphisms and some economic traits in cattle and water buffalo. There are studies about relations between *GH-Msp*I and *GH-Alu*I genotypes and some economic traits generally in cattle. However, especially because of extensive production system in water buffalo in Turkey, it was not possible to have data for milk or meat yield.

Arango et al. (2014) identified the significant association of genotypes at the *BGH-Msp*I locus with age at first service, first postpartum service, and first and second births in Holstein cows in the Antioquia province. Amiri et al. (2018) showed a significant effect of the *GH-Alu*I on the calving interval and the days open. The homozygous LL genotype has a favorable effect on calving interval and the days open compared with the LV and VV genotypes of the *GH-Msp*I locus.

Table 4 - Relationships between the *GH-Msp*I and *GH-Alu*I polymorphisms and some economic traits in cattle and water buffalo

Locus	Allele	Milk production trait			Reference
		Milk yield	Protein content	Fat content	
	(+)	↑	ND	ND	Høj et al., 1993; Lee et al., 1994
	(+)	↑	↑	↑	Yao et al., 1996
*GH-Msp*I	(–)	ND	ND	↑	Høj et al., 1993; Lee et al., 1994
	(–)	ND	ND	↑	Falaki et al., 1996
	(+,–)	ND	ND	ND	Özkan Ünal et al., 2015; in this study
	L	↑	ND	ND	Lucy et al., 1993
	V	ND	↑	↑	Sabour et al., 1997
*GH-Alu*I	V	ND	↑	↑	Zwierzchowski et al., 2002
	V	ND	ND	↑	Høj et al., 1993; Lee et al., 1994
	L,V	ND	ND	ND	Özkan Ünal et al., 2015; in this study

ND - not determined.
Arrows indicate increase (↑) or decrease (→).

5. Conclusions

Many studies have been carried out to prove the important relationship between *GH* genotypes and production traits. In this study, twenty new polymorphisms were found, which will provide information beneficial to improving growth traits in water buffalo based on marker-assisted selection. Therefore, in future studies, the possible relationships in water buffalo between these identified single nucleotide polymorphisms and some growth performance traits should be determined.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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References

Ahmadzadeh, M.; Rashidi, F.; Najafabadi, H. A.; Jaferian, A. and Eghbalsaied, S. 2019. Effects of genetic polymorphism in Pit1, GH, GHR and KCN3 on milk yield and body weight of Khuzestan (Iran) water buffaloes. Revista Colombiana de Ciencias Pecuarias 32:107-116. https://doi.org/10.17533/udea.rccp.v32n2a04

Akçay, A.; Akyüz, B. and Bayram, D. 2015. Determination of the *Alu*I polymorphism effect of bovine growth hormone gene on carcass traits in Zavot cattle with analysis of covariance. Turkish Journal of Veterinary and Animal Sciences 39:16-22. https://doi.org/10.3906/vet-1404-29

Akyüz, B.; Arslan, K.; Bayram, D. and İşcan, K. M. 2013. Allelic frequency of kappa-casein, growth hormone and prolactin gene in Holstein, Brown Swiss and Simmental cattle breeds in Turkey. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 19:439-444.

Amiri, S.; Jemmali, B.; Ferchichi, M. A.; Jeljeli, H.; Boulbaba, R. and Ben Gara, A. 2018. Assessment of growth hormone gene polymorphism effects on reproductive traits in Holstein dairy cattle in Tunisia. Archives Animal Breeding 61:481-489. https://doi.org/10.5194/aab-61-481-2018

Andreas, E.; Sumantri, C.; Nuraini, H.; Farajallah, A. and Anggraeni, A. 2010. Identification of GH|*Alu*I and GHR|*ALu*I genes polymorphisms in Indonesian buffalo. Journal of the Indonesian Tropical Animal Agriculture 35:215-221. https://doi.org/10.14710/jitaa.35.4.215-221

Arango G., J.; Echeverri Z., J. and López H., A. 2014. Association of the bovine growth hormone gene with Holstein cattle reproductive parameters. Revista MVZ Córdoba 19:4249-4258.

Ayuk, J. and Sheppard, M. C. 2006. Growth hormone and its disorders. Postgraduate Medical Journal 82:24-30. https://doi.org/10.1136/pgmj.2005.036087

Balogh, O.; Kovács, K.; Kulcsár, M.; Gáspárdy, A.; Zsolnai, A.; Kátai, L.; Pécsi, A.; Fésus, L.; Butler, W. R. and Huszenicza, G. 2009. A*lu*I polymorphism of the bovine growth hormone (GH) gene, resumption of ovarian cyclicity, milk production and loss of body condition at the onset of lactation in dairy cows. Theriogenology 71:553-559. https://doi.org/10.1016/j.theriogenology.2008.06.032

Carakushansky, M.; Whatmore, A. J.; Clayton, P. E.; Shalet, S. M.; Gleeson, H. K.; Price, D. A.; Levine, M. A. and Salvatori, R. 2003. A new missense mutation in the growth hormone-releasing hormone receptor gene in familial isolated GH deficiency. European Journal of Endocrinology 148:25-30. https://doi.org/10.1530/eje.0.1480025

Cosman, D.; Lyman, S. D.; Idzerda, R. L.; Beckmann, M. P.; Park, L. S.; Goodwin, R. G. and March, C. J. 1990. A new cytokine receptor superfamily. Trends in Biochemical Sciences 15:265-270. https://doi.org/10.1016/0968-0004(90)90051-C

Di Gerlando, R.; Sutera, A. M.; Mastrangelo, S.; Tolone, M.; Portolano, B.; Sottile, G.; Bagnato A.; Strillacci, M. G. and Sardina, M. T. 2019. Genome-wide association study between CNVs and milk production traits in Valle del Belice sheep. PLoS ONE 14:e0215204. https://doi.org/10.1371/journal.pone.0215204

Ermetin, O. 2017. Husbandry and sustainability of Water buffaloes in Turkey. Turkish Journal of Agriculture - Food Science and Technology 5:1673-1682. https://doi.org/10.24925/turjaf.v5i12.1673-1682.1639

Falaki, M.; Gengler, N.; Sneyers, M.; Prandi, A.; Massart, S.; Formigoni, A.; Burny, A.; Portetelle, D. and Renaville R. 1996. Relationships of polymorphisms for growth hormone and growth hormone receptor genes with milk production traits for Italian Holstein-Friesian bulls. Journal of Dairy Science 79:1446-1453. https://doi.org/10.3168/jds.S0022-0302(96)76503-7

FAO - Food and Agricultural Organization of the United Nations. 2017. FAOSTAT. FAO Statistics Division. Available at: <http://www.fao.org/faostat/en/#data/QA>. Accessed on: Apr. 16, 2019.

FAO - Food and Agriculture Organization of the United Nations. 2019. Available at: <http://www.fao.org/faostat/en/#data/QA>. Accessed on: Oct. 24, 2019.

Gil, F. M. M.; de Camargo, G. M. F.; Pablos de Souza, F. R.; Cardoso, D. F.; Fonseca, P. D. S.; Zetouni, L.; Braz, C. U.; Aspilcueta-Borguis, R. R. and Tonhati, H. 2013. Polymorphisms in the ghrelin gene and their associations with milk yield and quality in water buffaloes. Journal of Dairy Science 96:3326-3331. https://doi.org/10.3168/jds.2012-6362

Gordon, D. F.; Quick, D. P.; Erwin, C. R.; Donelson, J. E. and Maurer, R. A. 1983. Nucleotide sequence of the bovine growth hormone chromosomal gene. Molecular and Cellular Endocrinology 33:81-95. https://doi.org/10.1016/0303-7207(83)90058-8

Hall, T. 2011. Bioedit: Biological sequence alignment editor. Tom Hall, Ibis Biosciences, Carlsbad, CA.

HAYGEM. 2019. T.C. Tarım ve Orman Bakanlığı, Hayvancılık Genel Müdürlüğü, TUİK. Available at: <https://www.tarimorman.gov.tr/sgb/Belgeler/SagMenuVeriler/HAYGEM.pdf>. Accessed on: Nov. 12, 2019.

Høj, S.; Fredholm, M.; Larsen, N. J. and Nielsen, V. H. 1993. Growth hormone gene polymorphism associated with selection for milk fat production in lines of cattle. Animal Genetics 24:91-96. https://doi.org/10.1111/j.1365-2052.1993.tb00246.x

Hussain, D. A. 2015. Molecular Characterization of some productive traits in Mesopotamian buffaloes (*Bubalus bubalis*). European Journal of Molecular Biotechnology 8:80-87.

Hussain, D. A.; Ghareeb, A. M. and Salo, W. H. 2014. Evaluation of DNA polymorphism in bovine growth hormone gene by PCR-RFLP method. International Journal of Science and Nature 5:407-411.

Hussain, T.; Babar, M. E.; Ali, A.; Nadeem, A.; Rehman, Z. U.; Musthafa, M. M. and Marikar, F. M. M. T. 2017. Microsatellite based genetic variation among the buffalo breed populations in Pakistan. Journal of Veterinary Research 61:535-542. https://doi.org/10.1515/jvetres-2017-0057

Konca, M. A. and Akyüz, B. 2017. Investigation of growth hormone releasing hormone, growth hormone and prolactin hormone gene polymorphism in Anatolian water buffalo. Annals of Animal Science 17:1053-1062. https://doi.org/10.1515/aoas-2016-0100

Korkmaz Ağaoğlu, Ö. and Akyüz, B. 2013. Growth hormone gene polymorphism in four cattle breeds in Turkey. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 19:419-422.

Kour, A.; Chakravarty, A. K.; Raina, V. and Nag, P. 2018. Effect of intron 3 and exon 5 polymorphism in GH1 gene on milk production and milk composition traits in Karan Fries (Holstein Friesian Crossbred) cattle. International Journal of Livestock Research 8:81-87. https://doi.org/10.5455/ijlr.20180503102023

Lee, B. K.; Crooker, B. A.; Hansen, L. B. and Chester-Jones, H. 1994. Polymorphism in the third intron of somatotropin (bST) gene and its association with selection for milk yield in Holstein cows. Journal of Animal Science 72:316.

Lin, S.; Li, C; Li, C. and Zhang, X. 2018. Growth hormone receptor mutations related to individual dwarfism. International Journal of Molecular Sciences 19:1433. https://doi.org/10.3390/ijms19051433

Liu, J. J.; Liang, A. X.; Campanile, G.; Plastow, G.; Zhang, C.; Wang, Z.; Salzano, A.; Gasparrini, B.; Cassandro, M. and Yang, L. G. 2018. Genome-wide association studies to identify quantitative trait loci affecting milk production traits in water buffalo. Journal of Dairy Science 101:433-444. https://doi.org/10.3168/jds.2017-13246

Lucy, M. C.; Hauser, S. D.; Eppard, P. J.; Krivi, G. G.; Clark, J. H.; Bauman, D. E. and Collier, R J. 1993. Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production. Domestic Animal Endocrinology 10:325-333. https://doi.org/10.1016/0739-7240(93)90036-B

Meredith, B. K.; Kearney, F. J.; Finlay, E. K.; Bradley D. G.; Fahey, A. G.; Berry, D. P. and Lynn, D. J. 2012. Genome-wide associations for milk production and somatic cell score in Holstein-Friesian cattle in Ireland. BMC Genetics 13:21. https://doi.org/10.1186/1471-2156-13-21

Mishra, B. P.; Dubey, P. K.; Prakash, B.; Kathiravan, P.; Goyal, S.; Sadana, D. K.; Das, G. C.; Goswami, R. N.; Bhasin, V.; Joshi, B. K. and Kataria, R. S. 2015. Genetic analysis of river, swamp and hybrid buffaloes of north-east India throw new light on phylogeography of water buffalo (*Bubalus bubalis*). Journal of Animal Breeding and Genetics 132:454-466. https://doi.org/10.1111/jbg.12141

Mucha, S.; Mrode, R.; Coffey, M.; Kizilaslan, M.; Desire, S. and Conington, J. 2018. Genome-wide association study of conformation and milk yield in mixed-breed dairy goats. Journal of Dairy Science 101:2213-2225. https://doi.org/10.3168/jds.2017-12919

NCBI - National Center for Biotechnology Information. 2019a. Available at: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. Accessed on: Oct. 24, 2019.

NCBI - National Center for Biotechnology Information. 2019b. Available at: <https://www.ncbi.nlm.nih.gov/nuccore/NC_037547.1?report=GenBan>. Accessed on: Oct. 24, 2019.

NCBI - National Center for Biotechnology Information. 2019c. Available at: <https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_CAJ18232>. Accessed on: Oct. 24, 2019.

Öner, Y.; Pullu, M.; Akın, O. and Elmacı, C. 2011. Bursa bölgesinde yetiştirilen İsviçre Esmeri ve Siyah Alaca ırkı sığırlarda beta laktoglobulin (β-lg) ve büyüme hormonu (bGH) gen polimorfizmlerinin *HaeIII ve MspI* restriksiyon enzimleri kullanılarak incelenmesi. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 17:371-376.

Othman, E. O.; Abdel-Samad, M. F.; Abo El Maaty, N. A. and Sewify, K. M. 2012. Evaluation of DNA polymorphism in Egyption buffalo growth hormone and its receptor genes. Journal of Applied Biological Sciences 6:37-42.

Ozdemir, M.; Topal, M. and Aksakal, V. 2018. The relationships between performance traits and the bGH/Alu I and Pit-1/Hinf I polymorphisms in Holstein cows. Indian Journal of Animal Research 52:186-191. https://doi.org/10.18805/ijar.v0iOF.8495

Özkan Ünal, E.; Kepenek, E. Ş.; Dinc, H.; Özer, F.; Sönmez, G.; Togan, I. Z. and Soysal, M. I. 2015. Growth hormone (*GH*), prolactin (*PRL*), and diacylglycerol acyltransferase (*DGAT1*) gene polymorphisms in Turkish native cattle breeds. Turkish Journal of Zoology 39:734-748. https://doi.org/10.3906/zoo-1409-9

Özşensoy, Y. and Kara, H. 2019. Investigation of *GH* and *GHR Alu* I gene polymorphisms on meat yields in Anatolian water buffalo breed using PCR-RFLP method. Turkish Journal of Zoology 43:560-565. https://doi.org/10.3906/zoo-1907-44

Sabour, M. P.; Lin, C. Y. and Smith, C. 1997. Association of genetic variants of bovine growth hormone with milk production traits in Holstein cattle. Journal of Animal Breeding and Genetics 114:435-442. https://doi.org/10.1111/j.1439-0388.1997.tb00529.x

Schaeffer, L. R. 2006. Strategy for applying genome-wide selection in dairy cattle. Journal of Animal Breeding and Genetics 123:218-223. https://doi.org/10.1111/j.1439-0388.2006.00595.x

Schneider, J. F.; Rempel, L. A. and Rohrer, G. A. 2012. Genome-wide association study of swine farrowing traits. Part I: genetic and genomic parameter estimates. Journal of Animal Science 90:3353-3359. https://doi.org/10.2527/jas.2011-4729

Shi, D. S.; Wang, J.; Yang, Y.; Lu, F. H.; Li, X. P. and Liu, Q. Y. 2012. *DGAT1*, *GH*, *GHR*, *PRL* and *PRLR* polymorphism in Water Buffalo (*Bubalus bubalis*). Reproduction in Domestic Animals 47:328-334. https://doi.org/10.1111/j.1439-0531.2011.01876.x

Soysal, M. İ. 2013. Anatolian water buffaloes husbandry in Turkey. Buffalo Bulletin 32 (Special Issue 1):293-309.

Venturini, G. C.; Cardoso, D. F.; Baldi, F.; Freitas, A. C.; Aspilcueta-Borquis, R. R.; Santos, D. J. A.; Camargo, G. M. F.; Stafuzza, N. B.; Albuquerque, L. G. and Tonhati, H. 2014. Association between single-nucleotide polymorphisms and milk production traits in buffalo. Genetics and Molecular Research 13:10256-10268. https://doi.org/10.4238/2014.december.4.20

Woychik, R. P.; Camper, S. A.; Lyons, R. H.; Horowitz, S.; Goodwin, E. C. and Rottman, F. M. 1982. Cloning and nucleotide sequencing of the bovine growth hormone gene. Nucleic Acids Resesearch 10:7197-7210. https://doi.org/10.1093/nar/10.22.7197

Xie, L.; Luo, C.; Zhang, C.; Zhang, R.; Tang, J.; Nie, Q.; Ma, L.; Hu, X.; Li, N.; Da, Y. and Zhang, X. 2012. Genome-wide association study identified a narrow chromosome 1 region associated with chicken growth traits. PLoS One 7:e30910. https://doi.org/10.1371/journal.pone.0030910

Yao, J.; Aggrey, S. E.; Zadworny, D.; Hayes, J. F. and Kuhnlein, U. 1996. Sequence variations in the bovine growth hormone gene characterized by single-strand conformation polymorphism (SSCP) analysis and their association with milk production traits in Holsteins. Genetics 144:1809-1816.

Yardibi, H.; Hosturk, G. T.; Paya, I.; Kaygisiz, F.; Ciftioglu, G.; Mengi, A. and Oztabak, K. 2009. Associations of growth hormone gene polymorphisms with milk production traits in South Anatolian and East Anatolian red cattle. Journal of Animal and Veterinary Advances 8:1040-1044.

Zwierzchowski, L.; Krzyzewski, J.; Strzalkowska, N.; Siadkowska, E. and Ryniewicz, Z. 2002. Effects of polymorphism of growth hormone, pit-1 and leptin genes, cow's age, lactation stage and somatic cell count on milk yield and composition of Polish Black-and-White cows. Animal Science Papers and Reports 20:213-227.