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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\textbf{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 3 region of the \(GH\) gene. The partial DNA sequence of the \(GH\) gene in water buffaloes 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.

\textbf{Keywords:} biodiversity, breeding, DNA sequencing, growth traits

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 Ağaoglu 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; Hussein et al., 2014). Andreas et al. (2010) identified variation in GH/AluI locus in Indonesian buffalo. Shi et al. (2012) revealed genetic variation in GH/Alu and GH/MspI loci in Murrah river buffalo, Nili-ravi river buffalo, Chinese swamp buffalo, and Murrah-nili-swamp crossbreed buffalo. Hussein et al. (2014) investigated the GH/AluI 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, 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 ℃ 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’- GGACAGAGATAGCTCCATCCAG -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’- GACACCTACTCAGACAATGCAG -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 ((NH₄)₂SO₄, 200 μM dNTP, 2.0 mM MgCl₂, 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 ℃ for the initial denaturation, 35 cycles of amplification; 94 ℃ for 30 s, 58.1 ℃ annealing for 45 s,
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).

| Species | GH amino acid residue |
|---------|-----------------------|
| Buffalo | MMAAGPRTSLLALPCLLPWTSQVAGFPMASLSLFANAVLRQAQLHQLAAADTFKIER |
| Cattle  | ---------------------------------------------------------------G-------------------------------------------- |
| Goat    | ---------------------------------------------------------------G-------------------------------------------- |
| Sheep   | -------------------------TM----------------------------------G-------------------------------------------- |
| Red Deer| 1 -----------------------------------------E------------------G-------------------------------------------- |

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

| Species     | GH amino acid residue                   | Position |
|-------------|-----------------------------------------|----------|
| Buffalo     | MMAAGPRTSLLALPCLLPWTSQVAGFPMASLSLFANAVLRQAQLHQLAAADTFKIER | 60       |
| Cattle      | ---------------------------------------------------------------G-------------------------------------------- | 60       |
| Goat        | ---------------------------------------------------------------G-------------------------------------------- | 60       |
| Sheep       | -------------------------TM----------------------------------G-------------------------------------------- | 60       |
| Red Deer    | 1 -----------------------------------------E------------------G-------------------------------------------- | 60       |

| Species     | GH amino acid residue                   | Position |
|-------------|-----------------------------------------|----------|
| Buffalo     | TYPERRYSQIQTQVACFSETIPGTGKNEAQKSLSDDLRISSLISQSLPGPLFGE | 120      |
| Cattle      | ---------------------------------------------------------------G-------------------------------------------- | 120      |
| Goat        | ---------------------------------------------------------------G-------------------------------------------- | 120      |
| Sheep       | -------------------------TM----------------------------------G-------------------------------------------- | 120      |
| Red Deer    | 1 -----------------------------------------E------------------G-------------------------------------------- | 120      |
| MN266903    | 61 -----------------------------------------E------------------G-------------------------------------------- | 120      |

| Species     | GH amino acid residue                   | Position |
|-------------|-----------------------------------------|----------|
| Buffalo     | RVFTNSLVGTSRVEKLDLEGHALMREGDTPRAGQLR----TYDKFDTNMRSDDA | 180      |
| Cattle      | ---------------------------------------------------------------V-------------------------------------------- | 180      |
| Goat        | ---------------------------------------------------------------V-------------------------------------------- | 180      |
| Sheep       | ---------------------------------------------------------------V-------------------------------------------- | 180      |
| Red Deer    | 121 -----------------------------------------V-------------------------------------------- | 180      |
| MN530974    | 121 -----------------------------------------R-------------------------------------------- | 180      |

| Species     | GH amino acid residue                   | Position |
|-------------|-----------------------------------------|----------|
| Buffalo     | LLKNYGLLSCFRKDLHKTEYLRVMNRFFGAECAF      | 217      |
| Cattle      | 181 -----------------------------------------R-------------------------------------------- | 217      |
| Goat        | 181 -----------------------------------------R-------------------------------------------- | 217      |
| Sheep       | 181 -----------------------------------------R-------------------------------------------- | 217      |
| Red Deer    | 181 -----------------------------------------R-------------------------------------------- | 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 AluI regions from GH gene on water buffalo in Turkey

| Locus       | Position | Nucleotide change | Genbank accession number | Type of mutation |
|-------------|----------|-------------------|--------------------------|-----------------|
| GH/Alu      | 2108     | C                  | Y (0.006)                | MN30973         | Transition    |
|             | 2139-42  | AGCT               | -                        | All Sequences   | Deletion      |
|             | 2142     | T                  | D (1.000)                | MN30974         | Transversion  |
|             | 2149     | T/C                | T (0.013)                | MN30975-6       | Transition    |
|             | 2178     | A/A                | R (0.006)                | MN30974         | Transition    |
|             | 2272     | G                  | G (0.013)                | MN30975-6       | Transition    |
|             | 2275     | A/G                | A (0.013)                | MN30975-6       | Transition    |
|             | 2291     | A/C                | M (0.006)                | MN30976         | Transition    |
|             | 2329     | T                  | T (0.013)                | MN30975-6       | Transition    |
|             | 2358     | T                  | T (1.000)                | MN30975-6       | Deletion      |
|             | 2380     | T                  | T (0.013)                | MN30975-6       | Insertion     |
| GH/MspI     | 1427     | T                  | T (0.013)                | MN266903        | Transition    |
|             | 1448     | G                  | G (0.013)                | MN266905        | Transition    |
|             | 1457     | T                  | T (0.020)                | MN266905-7      | Transition    |
|             | 1475     | A                  | A (0.013)                | MN266905-7      | Transition    |
|             | 1485     | A                  | A/C (0.006)              | MN266905        | Transition    |
|             | 1490     | C                  | C/A (0.006)              | MN266905        | Transition    |
|             | 1520     | C                  | C/T (0.006)              | MN266905        | Transition    |
|             | 1541     | -                  | T (1.000)                | MN266903        | Insertion     |
|             | 1543     | C                  | C/T (0.006)              | MN266905        | Transition    |
|             | 1551     | -                  | G (1.000)                | MN266903        | Insertion     |
|             | 1559     | C                  | C/T (0.006)              | MN266905        | Transition    |
|             | 1560     | G                  | A/G (0.013)              | MN266907        | Transition    |
|             | 1571     | C                  | C/T (0.006)              | MN266905        | Transition    |
|             | 1580     | G                  | C/G/A (0.006)            | MN266905        | Transition    |
|             | 1584     | G                  | A/G (0.013)              | MN266907        | Transition    |
|             | 1585     | T                  | A/T/G (0.006)            | MN266905        | Transition    |
|             | 1613     | G                  | G/T (0.006)              | MN266905        | Transition    |
|             | 1616     | C                  | C/T (0.006)              | MN266905        | Transition    |
|             | 1658     | A                  | A/G (0.020)              | MN266905        | Transition    |
|             | 1665     | A                  | A/G (0.006)              | MN266905        | Transition    |
|             | 1666     | G                  | A (1.000)                | MN266903        | Transition    |
|             | 1667     | G                  | G (1.000)                | MN266903        | Transition    |
|             | 1685     | G                  | A (0.006)                | MN266905        | Transition    |
|             | 1692     | C                  | 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.
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Figure 2 - Nucleotide changes seen in the studied samples of the GH/AluI loci at positions a) 2108. (Y), b) 2142. (W), c) 2178. (R), and d) 2291. (M) of the cattle GH gene (M57764).
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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 MspI 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/MspI 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 MspI and Alu I endonuclease restriction sites are shown in Table 3.

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

| Locus | Genotype Frequency | Allele frequency |
|-------|--------------------|------------------|
|       | CC | CT | TT | C | T |
| MspI  | Observation | 150.00 | 0.00 | 0.00 | 1.00 | 0.00 |
|       | Expectation | 150.00 | 0.00 | 0.00 | 1.00 | 0.00 |
|       | Frequency | 1.00 | 0.00 | 0.00 | 1.00 | 0.00 |
| Alu I | Observation | 147.00 | 3.00 | 0.00 | 0.99 | 0.01 |
|       | Expectation | 147.02 | 2.97 | 0.01 | 0.99 | 0.01 |
|       | Frequency | 0.020 | 0.02 | 0.00 | 0.02 | 0.00 |

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

Figure 4 - Position of primers based on GenBank (Accession number KC107770) and position of Alu I endonuclease restriction site.
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/AluI gene at 432 bp located on intron 4, exon 4-5, and found that the GH/AluI 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 et 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-MspI 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-MspI and GH-AluI polymorphisms and some economic traits in cattle and water buffalo. There are studies about relations between GH-MspI and GH-AluI 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-MspI 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-AluI 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-MspI locus.
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

Data curation: E. Özkan Ünal. Formal analysis: E. Özkan Ünal. Investigation: R. Işık and M.İ. Soysal. Methodology: E. Özkan Ünal and M.İ. Soysal. Project administration: E. Özkan Ünal. Software: E. Özkan Ünal and R. Işık. Supervision: M.İ. Soysal. Validation: M.İ. Soysal. Writing-original draft: E. Özkan Ünal, R. Işık and M.İ. Soysal. Writing-review & editing: E. Özkan Ünal, R. Işık and M.İ. Soysal.

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Table 4 - Relationships between the GH-MspI and GH-AluI polymorphisms and some economic traits in cattle and water buffalo

| Locus | Allele | Milk production trait | Reference |
|-------|-------|------------------------|-----------|
|       |       | Milk yield | Protein content | Fat content |
| GH-MspI | (+) | ↑ | ND | ND | Høj et al., 1993; Lee et al., 1994 |
|       | (+) | ↑ | ↑ | ↑ | Yao et al., 1996 |
|       | (-) | ND | ND | ↑ | Høj et al., 1993; Lee et al., 1994 |
|       | (+) | ND | ND | ND | Falakiet al., 1996 |
|       | (+) | ND | ND | ND | Özkan Ünal et al., 2015 | in this study |
|       | L | ↑ | ND | ND | Lucy et al., 1993 |
|       | V | ↑ | ↑ | ↑ | Sabour et al., 1997 |
| GH-AluI | V | ↑ | ↑ | ↑ | Zwierzchowski et al., 2002 |
|       | V | ND | ↑ | ↑ | Sabour et al., 1997 |
|       | LV | ND | ND | ND | Özkan Ünal et al., 2015 | in this study |

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