Original article

Detection a novel genetic variant of the Myf5 gene in Turkish Anatolian Water Buffalo

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Abstract. Myf5 gene belongs to myogenic regulatory factors (MRFs) families and has an active role in muscle differentiation and the development of skeletal muscle cells. The study aimed to detect genetic variants of the Myf5 gene in Anatolian buffaloes. PCR-RFLP and DNA sequencing methods were used to detect SNP regions of the Myf5 gene. Seven restriction enzymes were used to identify the polymorphic regions. Six enzymes (*RsaI, SspI, DraI, HinfI, PstI, and EcoRI*) showed monomorphic regions and only, *BsuRI* enzyme showed a polymorphic region in the intron 2 site. It was detected a novel SNP in the C14553A site in the second intron of the Myf5 gene in Anatolian buffaloes. These novel findings may be used as a genetic marker and contribute to the performance of buffaloes in the future.

Key words: Anatolian Buffalo, Myf5, sequencing, SNPs, *BsuRI*

Introduction

BBuffaloes are the second source of milk production after cows. Buffalo milk has distinct nutritional qualities, the most important of which is the high-fat content and the white milk color that consumers prefer. Buffaloes are more endurance to environmental conditions and resistant to diseases than other farm animals (1-3). The number of buffaloes population in the world is about 207 million heads. Asia ranks first at about 97 percent; 2 percent in Africa, primarily Egypt; 0.7 percent in South America; and less than 0.2 percent in Europe and Australia. Pakistan, China, India, Nepal, and Egypt have many of buffaloes (4). The buffalo in Turkey originated from the Mediterranean buffalo, a subspecies of river buffalo, defined as Anatolian Water Buffalo (Bubalus bubalis Linnaeus, 1758). In 2004 it was registered as a native breed by the Breed Registration Committee (5-7). According to the Turkish Statistical Institute (TÜİK), in the 2020 year, the number of buffaloes in Turkey was 188,771 heads, milk yield was 70,341 tons, and meat production was 73 tons. Anatolian buffalo are bred for three purposes: milk, meat and work. In Turkey, kaymak (creamy), yogurt, cheese and ice cream are produced from buffalo milk, and sucuk (sujuk) is made from buffalo meat. The productive characteristics of the Anatolian buffalo are; live weight in adult males and females is 550-600 and 400-450 kg; respectively, average birth weight is 30 kg, daily live weight gain 550-600 g, lactation milk yield 800-1000 kg, lactation period 200-250 days and the fat rate in milk is around 6-8% (7). With the development of molecular genetic techniques, it has become possible to improve the traits of farm animals. Molecular markers allow the detection of variations or polymorphisms that exist among individuals in the population for specific regions of DNA.

Different markers have been used successfully for these purposes (8-10). With the development of molecular biology, it has become possible to identify candidate genes associated with economic traits in farm animals. In addition, these genes are used as genetic markers in marker-assisted selection (MAS) programs (11-13). Muscle formation is a multistep process

involving commitment, proliferation, and specification during embryo growth to postnatal maturation and function and is controlled by the myogenic determination (MyoD) gene family (14-16). MyoD contains four genes; Myf3, Myf4, Myf5, and Myf6. The Myogenic factor 5 (Myf5) gene belongs to the myogenic regulatory factors families plays a vital function in the gene expression related to muscle, differentiation, and forming of muscle fibers, especially the development of skeletal muscle (17-21). QTL position of the Myf5 locus mapped 0 and 30 cM within BTA5. The length of the bovine Myf5 gene is 3236 bp and contains two introns and three exons (22-24).

In Canadian cattle, an association was determined between the Myf5 gene polymorphism and growth traits (25); average daily gain (ADG) and growth traits in Han woo breeds (22); growth traits in Qinchuan breeds (23), and carcass and growth traits in Korean Han woo breeds (19; 26). Various studies detected significant effects of the Myf5 gene on carcass traits instead of meat qualities traits in pigs (27-29). However, Khang and Ngu (2013) showed a significant association between Myf5/Hin1II with loin weight, compression force, dressing percentage and meat pH45min in Mong Cai breeds. In contrast, it identified an association between Myf5/Hsp92II polymorphisms, meat moisture content, and intramuscular fat (20). However, the genetic polymorphisms of the Myf5 gene were investigated in various mammal species. However, no study was conducted to investigate Myf5 gene polymorphisms in buffaloes. This study aims to detect the novel variants of the Myf5 gene in Anatolian Water Buffaloes.

Materials and methods

Animal Material

A total of 80 animals were collected from two native Anatolian Water Buffalo breeds including forty-one animals from Kütahya and nineteen animals from Konya provinces in Turkey. Blood samples were taken from the jugular vein and placed into 10 mL tubes containing EDTA and stored at -20 oC.

DNA extraction, PCR assay, genotyping and DNA sequencing

Genomic DNA was extracted from bloods. QuickGene DNA whole blood kit S (DB-S) (KURABO, JAPAN) was used to DNA extraction. After extraction, the DNA concentration of all samples was measured with the Nanodrop spectrophotometer (ND1000; NanoDrop Technologies, USA). The primer sequences and PCR conditions for the Myf5 gene locus are shown in Table 1.

The PCR mixture (10 μL) contained the following components and conditions; genomic DNA, 5 μmol L–1 DreamTaq Green PCR Master Mix (2X; Thermo Scientific), 0.3 μmol L–1 of each primer and 3.4 μmol L–1 bdH2O. The RFLP method was used to determine for polymorphisms in the Myf5 gene locus. A 20 μL of the PCR products was digested with 5 U of RsaI, SspI, BsuRI, DraI, HinfI, PstI and EcoRI fast digest restriction enzymes (Thermo Scientific) 5-30 min at 65 °C, 37 °C and 37 °C in incubator and then the reaction being stopped by 65 °C. The restriction fragments were separated in 2% agarose gel and stained with ethidium bromide The gel was viewed under UV light, and scored in a gel documentation system. The

Table 1. The primer sequences of Myf5 gene locus

Gene	Primer sequences	Fragment length	PCR conditions	Reference
Myf5	5'-GATAGCTGGCTGTGAATGAT-3' 5'-CTGGCAACTGGGGAGAGAGA-3'	1190 bp	94°C 3m, 94°C 30s, 60°C 40s, 72°C 1m, 39 cycles 72°C 10m	Barth et al. (1993) (GenBank accession No. M95684)

digest products were sequenced in both directions for the identification of the SNPs.

Statistical analysis

The Chi-square test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium was carried out by PopGene Version 1.32. The sequences analysis was made in MEGA version 6.0.

Results

1190 bp was amplified of PCR product. RFLP-PCR technology was used to identify SNP regions at the Myf5 locus. Seven restriction enzymes were used to detect a novel polymorphism in the intron 2 region of the Myf5 gene in Anatolian Water Buffalo. No polymorphic regions were detected when using *RsaI, SspI, DraI, HinfI, PstI* and *EcoRI* enzymes and it was a monomorphic restriction pattern (Figure 1). A polymorphism region was detected when using the BsuRI enzyme. Two different genotypes have been identified (Figure 2). Sequencing results showed a point mutation at the C14553A site (Figure 3).

Discussion

The Myf5 gene plays an essential role in the growth and development of livestock. The reason for choosing this locus of the Myf5 gene is because of its association with growth traits in different cattle breeds. There are

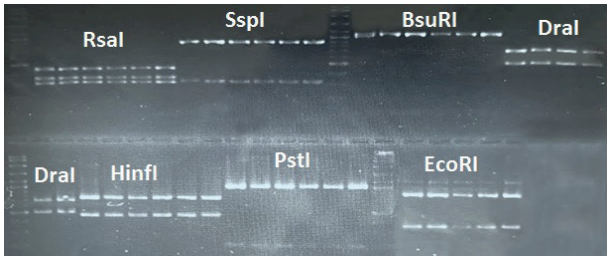

Figure 1. Digest the Myf5 gene locus with different restriction enzymes

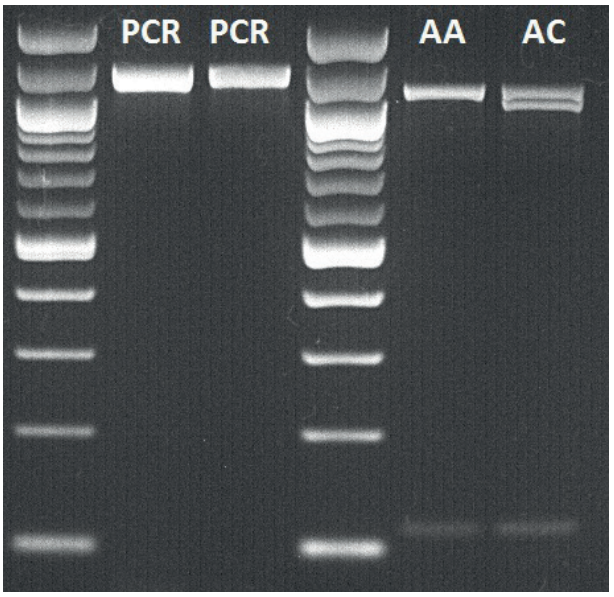

Figure 2. Digest the Myf5 gene locus with *BsuRI* enzyme

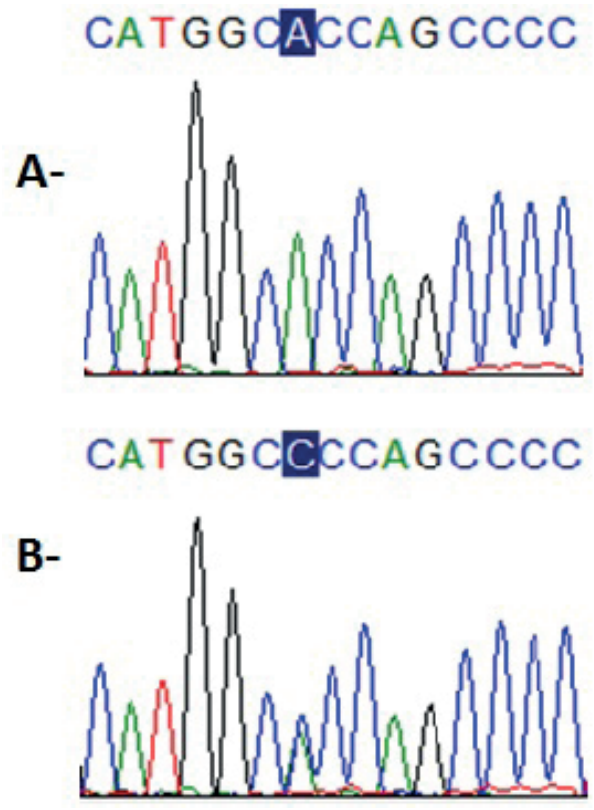

Figure 3. A: Sequencing map of novel SNPs in Myf5 gene, B: Sequencing map of Myf5 gene

few studies that characterizing the Myf5 gene in buffaloes. Daldaban et al. (2020) determined the genetic characterization of the Myf5 gene in Anatolian water buffalo. Two genotypes were detected with allele and genotype frequency 0.23(AG), 0.77(GG); 0.12(A), 0.88(G). Zhang et al. (2007) showed significant effects of the Myf5 gene on height at hip cross and withers height in Qinchuan breeds ($p<0.05$). Nasr et al. (2016) observed an association between the Myf5 gene and body weight in Friesian bull calves. Fadhil and Zülkadir (2021) reported an association between genes and fattening performance in Brown Swiss and Holstein cattle breeds. Wang et al. (2017) showed effect variation of Myf5 gene in intron 2 site on carcass lean meat yield in New Zealand Romney sheep. Liu et al. (2007) found a significant association between Mfy5 gene in intron 1 and 2 locus with biceps femoris marbling score ($p<0.01$), longissimus dorsi Water moisture content ($p<0.01$), water holding capacity ($p<0.05$), drip loss rate ($p<0.05$), biceps femoris meat color value ($p<0.05$) and longissimus dorsi intramuscular fat percentage ($p<0.01$).

Conclusion

Several studies have been reported in cattle, sheep, and pigs for the intron site of the Myf5 gene. However, a few studies described the Myf5 gene in buffaloes. In this study, novel SNPs were detected in the Myf5 gene at the intron 2 site, which can change the protein's activity and can use as genetic markers in animal breeding programs. However, further association studies are necessary to verify whether this SNP is associated with growth or other traits in the Turkish Anatolian Water Buffalo.

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Conflict of interest: There is no conflict of interest

References

1. Becskei Z, Savić M, Ćirković D, et al. Assessment of water buffalo milk and traditional milk products in a sustainable production system. Sustainability 2020; 12: 1-13.
2. Khan S, Qureshi M S, Ahmed I, Shah S M. Milk composition and yield changes with advancing pregnancy in dairy buffaloes (Bubalus bubalis). Turk. J. Vet. Anim. Sci 2011; 35: 375-380.
3. Soysal M, Ozkan E, Kok S, et al. Genetic characterization of indigenous anatolian water buffalo breed using microsatellite dna markers. Ital. J. Anim. Sci 2007; 6: 409-412.
4. FAO, 2021. Food and Agricultural Organization of the United Nations, Buffaloes. Available on: http://www.fao.org/dairy-production-products/production/dairy-animals/buffaloes/en/.
5. Değirmencioğlu T. Possibilities to Reduce Thermal Stress in Water Buffalo [Bubalus bubalis (Linnaeus, 1758)]. AARI 2020; 30: 117-123.
6. Karlı B, Gül M, Akpınar M G, Taşcıoğlu Y, Bozkurt Y, Şirikçi B S. Analysis of economic structure in water buffalo breeding by geographical regions in Turkey. Buffalo Bull 2021; 40: 135-150.
7. Kelgökmen İ, Ünal N. Some morphometric traits of Anatolian Buffaloes. Lalahan Hay. Araşt. Enst. Derg 2015; 55: 50-55.
8. Aytekin I, Bayraktar M. Bovine SMO gene polymorphism in Anatolian Black Cattle and Anatolian Water Buffaloes. Adü Ziraat Derg 2021; 18: 29-32.
9. Aytekin İ, Bayraktar M, Sakar Ç M, Ünal İ. Association between MYLK4 gene polymorphism and growth traits at different age stages in Anatolian black cattle. Anim. Biotechnol 2020; 31: 555-560.
10. Ogorevc J, Kunej T, Razpet A, Dovc P. Database of cattle candidate genes and genetic markers for milk production and mastitis. Anim. Genet 2009; 40: 832-851.
11. Aytekin I, Boztepe S. Associations of Pit-1 gene polymorphism with milk yield and composition traits in brown swiss cattle. J Anim Plant Sci 2013; 23: 1281-1289.
12. Aytekin I, Ozdil F, Zulkadir U, Boztepe S, Sariyel V. Evaluation of ISSR markers for genetic diversity analysis in Anatolian water buffaloes. J. Sci. Food Agric 2011; 91: 1957-1962.
13. Rezende F, Ferraz J, Eler J, Silva R, Mattos E, Ibáñez-Escriche N. Study of using marker assisted selection on a beef cattle breeding program by model comparison. Livest. Sci 2012; 147: 40-48.
14. Bentzinger C F, Wang Y X, Rudnicki M A. Building muscle: molecular regulation of myogenesis. Cold Spring Harb. Perspect. Biol 2012; 4: 1-16.
15. Genxi Z, Ying T, Tao Z, Jinyu W, Yongjuan W. Expression profiles and association analysis with growth traits of the MyoG and Myf5 genes in the Jinghai yellow chicken. Mol. Biol. Rep 2014; 41: 7331-7338.

16. Kunhareang S, Zhou H, Hickford J G. Allelic variation in the porcine MYF5 gene detected by PCR–SSCP. Mol. Biotechnol 2009; 41: 208-212.
17. Fadhil M, Zülkadir U. Association between polymorphisms of Myf5, MSTN and CAST genes and fattening performance in Brown Swiss and Holstein cattle breeds. Anim. Biotechnol 2021; 32: 121-129.
18. Saputra E, Ulum M, Jakaria J. Association of SNP g. 643G> A of MYF5 gene polymorphism with body weight and body measurements in Bali cattle. J. Indones. Trop. Anim. Agric 2020; 45: 1-6.
19. Ujan J, Zan L, Ujan S, Wang H, 2011. Association between polymorphism of MYF5 gene with meat quality traits in indigenous Chinese cattle breeds. International Conference on Asia Agriculture and Animal. IACSIT, Singapore, pp. 50-55.
20. Wang J, Hu Y, Elzo M A, et al. Genetic effect of Myf5 gene in rabbit meat quality traits. J. Genet 2017; 96: 673-679.
21. Hernández-Hernández J M, García-González E G, Brun C E, Rudnicki M A. The myogenic regulatory factors, determinants of muscle development, cell identity and regeneration. Semin. Cell Dev. Biol 2017; 72: 10-18.
22. Chung E, Kim W. Association of SNP marker in IGF-I and MYF5 candidate genes with growth traits in Korean cattle. Asian-australas. J. Anim. Sci 2005; 18: 1061-1065.
23. Zhang R, Chen H, Lei C, et al. Association between polymorphisms of MSTN and MYF5 genes and growth traits in three Chinese cattle breeds. Asian-australas. J. Anim. Sci 2007; 20: 1798-1804.
24. 24. Zhao C, Raza S H A, Khan R, et al. Genetic variants in MYF5 affected growth traits and beef quality traits in Chinese Qinchuan cattle. Genomics 2020; 112: 2804-2812.
25. Li C, Basarab J, Snelling W, et al. Assessment of positional candidate genes myf 5 and igf 1 for growth on bovine chromosome 5 in commercial lines of Bos taurus. J. Anim. Sci 2004; 82: 1-7.
26. Bhuiyan M, Kim N, Cho Y, et al. Identification of SNPs in MYOD gene family and their associations with carcass traits in cattle. Livest. Sci 2009; 126: 292-297.
27. Cieslak D, Kuryl J, Kapelanski W, Pierzchala M, Grajewska S, Bocian M. Relationship between genotypes at at MYOG, MYF3 and MYF5 loci and carcass meat and fat deposition traits in pigs. Anim. Sci. Pap. Rep 2002; 20: 79-92.
28. Liu M, Peng J, Xu D, et al. Associations of MYF5 gene polymorphisms with meat quality traits in different domestic pig (Sus scrofa) populations. Genet. Mol 2007; 30: 370-374.
29. Te Pas M, Harders F, Soumillion A, Born L, Buist W. Genetic variation at the porcine MYF-5 gene locus. Lack of association with meat production traits. Mamm. Genome 1999; 10: 123-127.
30. Khang N T K, Ngu N T. Effects of myogenic factor 5 (MYF5) gene on carcass and meat quality of Mong Cai pigs. Thai J. Vet. Med 2013; 43: 213-218.
31. Daldaban F, Arslan K, Aksel E G, Akyüz B. Polymorphism of the STAT5A and MYF-5 genes in Anatolian water buffalo. Turk. J. Vet. Anim. Sci 2020; 44: 284-289.
32. Nasr S, Ateya A, Sadek K, Radwan H. TaqI polymorphism in MYF5gene and its association with body weight in Friesian bull calves. Asian. J. Anim. Vet. Adv 2016; 11: 429-433.
33. Wang J, Zhou H, Forrest R H, et al. Variation in the ovine MYF5 gene and its effect on carcass lean meat yield in New Zealand Romney sheep. Meat Sci 2017; 131: 146-151.

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