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MYOSTATIN GENE POLYMORPHISMS DO NOT DETERMINE MUSCLE HYPERTROPHY IN BUFFALOES: A CASE REPORT

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ABSTRACT

The aim of the study was to detect variations in the exonic and intronic regions of the myostatin gene in buffaloes with different body conformations. The regions of the gene were analyzed using the PCR-sequencing technique comparing an animal with muscle hypertrophy and ten animals with normal musculature. The hypertrophied buffalo sequence was compared to the others and no exclusive genotype was identified. It suggests that the found *MSTN* variants are not singly associated to muscle hypertrophy in buffaloes.

Keywords: *Bubalus bubalis*, buffaloes, double musculature, GDF8, MSTN, Murrah

INTRODUCTION

The Murrah buffalo is originally from Northwest India. It is one of the four buffalo breeds raised in Brazil being the one with the biggest population in the country (da Silva *et al*., 2021; ABCB, 2023). The breed is distinguished from the others due to the small, curled horns (oval or triangular section). Murrah buffalo is considered a dual-purpose breed and is under genetic evaluation in Brazil (da Silva *et al*., 2021; ABCB, 2023).

The myostatin gene (*MSTN*), also known as *GDF8*, is a negative regulator of muscle tissue growth (McPherron *et al*., 1997; Aiello *et al*., 2018). In buffaloes, *MSTN* consists of three exons and two introns (Tantia *et al*., 2007). Polymorphisms in the myostatin gene have been associated with the phenotype of muscle hypertrophy in different domestic animals (Aiello *et al*., 2018; Jakaria *et al*., 2021). We investigated polymorphisms in the coding and intronic regions of the gene in buffaloes with muscle hypertrophy in order to identify genetic variants that influence this phenotype.

MATERIALS AND METHODS

Eleven Murrah buffaloes were studied, including one with the muscle hypertrophy

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phenotype and 10 unrelated animals with "normal" musculature (Figure 1). The Ethics Committee on Animal Use of EMVZ-UFBA approved the project (81/2018). The frequency of occurrence of the phenotype in the population was uncertain. Genomic DNA was extracted from hair follicle samples using the DNA NucleoSpin Tissue kit (Macherey-Nagel). Primers were designed for PCR amplification based on the sequences DQ091762.1 and NC_037546.1 available in NCBI Genbank (https://www.ncbi.nlm.nih.gov). Nine primer pairs (Table 1) were designed using online software Primer 3 Plus (https://primer3plus.com/cgi-bin/dev/primer3plus.cgi) to amplify exons 1, 2 and 3 and introns 1 and 2 of the MSTN.

The PCR mixture contained 100 ng DNA, 1.0 µl of each primer (10 mM), and 12.5 µl Taq mix (dNTPs, buffer, $MgCl_2$ and Taq polymerase (GoTaq Colorless Master Mix, 2X - final concentration in the 1X reaction). For amplification, an initial denaturation step was performed at 95°C for 3 minutes, followed by 35 cycles at 95°C for 1 minute, annealing for 54 to 57°C for 1 minute, 72°C for 1 minute, and a final extension cycle at 72°C for 10 minutes.

The PCR products were purified by precipitation with 20% polyethylene glycol (PEG). Both DNA strands were sequenced by the same forward and reverse primers, using the BigDye v3.1 sequencing kit (Applied Biosystems, Foster City, CA, USA) in a 3500xL DNA sequencer (Applied Bio-systems) according to manufacturer instructions. The sequences were deposited in GenBank under the accession number OQ378332.

RESULTS AND DISCUSSIONS

Analysis of the sequences of the *MSTN* gene of all buffaloes revealed two SNPs and two indels (all in intron 1) across the animals, not previously detected. The hypertrophied buffalo sequence was compared to the others and no exclusive genotype was identified. It suggests that the found MSTN variants are not singly associated to muscle hypertrophy in buffaloes. Páez *et al.* (2021) also observed absence of variants associated to the phenotype, studying exons 2 and 3 of MSTN in buffaloes. The polymorphisms herein identified and not the same identified by Páez *et al*. (2021), since they are intronic. The study of other candidate genes is necessary to identify the polymorphisms that cause the muscle hypertrophy phenotype in buffaloes. Despite its uncertain frequency, the phenotype might be interesting for meat production in buffaloes because it is not associated with negative conditions as observed in cattle. The identification of polymorphism(s) that affect this phenotype as well as its gene action is important to perform selection. The present results suggest that muscle hypertrophy in buffaloes is not singly associated to myostatin gene variants, differently from other livestock species. Other candidate genes should be studied, so molecular markers should be developed for selection. The research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnologógico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Universidade Federal da Bahia (UFBA).

Table 1. Primers are used for amplification of exons 1, 2 and 3 and introns 1 and 2 of the myostatin gene in Murrah buffaloes.

Target region	Primer sequence	Annealing temperature (°C)	Size of PCR product (bp)
Exon 1	Forward 5'-CTG GTG TGG CAA GTT GTC TC-3' and reverse 5'-GGC TTC AAC CTC TAC AGA TTT CT-3'	54	962
Exon 2	Forward 5'-TGG AGG TGT TCG TTC GTT TT-3' and reverse 5'-TGT GTT GTT GGG TGT GTA CT-3'	54	811
Exon 3	Forward 5'-GCT ACT GTA GAC TTT TGA GCC A-3' and reverse 5'-TCA CCA GAA GAC AAG GAG AAT T-3'	54	880
Intron 1	Forward 5'- GTA AGG AGG AGG GGG AAG AG-3' and reverse 5'-CAT GGT CAG GGT ATA AGT GGA AC-3'	54	632
Intron 1	Forward 5'- CAA AGT TCC ACT TAT ACC CTG ACC-3' and reverse 5'-CTG TCA TTG TAA GCA GAA GCA C-3'	54	495
Intron 1	Forward 5'- GCT TCT GCT TAC AAT GAC AGC C-3' and reverse 5'-TGA AAA ACG AAC GAA CAC CTC C-3'	55	618
*Intron 2	Forward 5'-ACC CAA CAA AAG TAG GTG TCC-3' and reverse 5'-AGC ATA AAT AAG CCA GAA GAG TGA G-3'	56	692
Intron 2	Forward 5'-CAC TCT TCT GGC TTA TTT ATG CTT G-3' and reverse 5'-ACT CAT TCA TCC TCC ATT CAC TTG-3'	57	789
Intron 2	Forward 5'-CCA CTA TCT TC ATC ACT CTA CCT TC-3' and reverse 5'-TCC CAA AAC ACT CTC CTA CCT C-3'	55	532

*Amplicon purified from agaroses gel (purified with NZYTECH kit).

Figure 1. A: Buffalo with muscle hypertrophy; B: Two buffaloes side by side, one with normal musculature and the other with muscle hypertrophy.

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