Notas Científicas

Mitochondrial DNA of Nellore and European x Nellore crossing cattle of high performance

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Abstract – The objective of this work was to evaluate, through a polymorphism in the ND5 gene of the bovine mitochondrial DNA, the frequency of *Bos taurus indicus* mtDNA individuals in a sample of Nellore purebred origin animals (n = 69) and crossbred animals originated from crosses of European sires and Nellore purebred origin females (n = 275). Only 2.26% (8/354) of the animals presented *Bos taurus indicus* mtDNA. The high frequency of *Bos taurus taurus* mtDNA in these animals can be a consequence of selection, once the animals studied are originated from selected lineages of high performance for meat production.

Index terms: *Bos taurus indicus*, *Bos taurus taurus*, beef cattle, DNA polymorphism.

DNA mitocondrial de bovinos Nelore e cruzados Europeu x Nelore de alto desempenho

Resumo – O objetivo deste trabalho foi avaliar, por meio de um polimorfismo no gene ND5 do DNA mitocondrial de bovinos, a porcentagem de indivíduos portadores de mtDNA *Bos taurus indicus* em animais Nelore PO (n = 69) e em animais provenientes do cruzamento entre machos europeus e fêmeas Nelore PO (n = 275). Apenas 2,26% (8/354) dos animais apresentaram mtDNA *Bos taurus indicus.* A alta freqüência de mtDNA *Bos taurus taurus* nesses animais pode ser reflexo de seleção, uma vez que os animais estudados se originam de linhagens selecionadas para alto desempenho de produção de carne.

Termos para indexação: *Bos taurus indicus*, *Bos taurus taurus*, gado de corte, polimorfismo de DNA.

There are two major groups of cattle, *Bos indicus* or Zebu (humped) and *Bos taurus* or European (humpless), according to the classical Linnean nomenclature. However, the complete interfertility between *B. indicus* and *B. taurus* led several authors to consider both as subspecies. Currently, they are usually separated into *Bos taurus indicus* and *Bos taurus taurus* (Issa et al., 2006).

A matrilineal European participation in Zebu cattle, since its introduction in American lands, has been reported. This hybridization is confirmed by the major contribution of *Bos taurus taurus* mitochondrial DNA in these animals (Ripamonte, 2002). It is likely that mtDNA polymorphism played a significant role through natural selection in the adaptation of different cattle groups to regional environmental conditions (Meirelles et al., 2001). Moreover, bovine dairy and beef production traits have shown variation in different maternal lineages, likely originated from polymorphic mitochondrial genotypes (Tess et al., 1987; Mannen et al., 1998).

The objective of this work was to estimate the frequency of individuals with *Bos taurus indicus* mtDNA in a sample of animals with Nellore maternal lineages of high performance for meat production.

Blood was collected from 354 bulls belonging to three different genetic groups: 79 Nellore purebred origin, and 275 crossbred animals originated from crosses of Simmental (n = 30) and Angus (n = 245) sires with Nellore purebred origin females. Genomic DNA was extracted from a 300 μL aliquot of total blood using the Genomic Prep Blood DNA Isolation kit (Amersham Biosciences, Piscataway, NJ, USA). The amount and integrity of the DNA were

determined on 0.8% agarose gel. For the determination of *Bos taurus taurus* or *Bos taurus indicus* mtDNA, a 755 bp fragment – nucleotide 11.770 to 12.525 according to Anderson et al. (1982) – of ND5 gene of the mitochondrial genome was amplified and digested with *Hind*III restriction enzyme (ND5/*Hind*III polymorphism).

The amplification of mtDNA was performed using primers 5' – CCCAACGAGGAAAATATACC – 3' and 5' – GGAAGAGGTTGTTTGCGGTT – 3' designed based on Genbank sequence: Gi 5834939. Each PCR was performed in a final volume of 25 µL, with the amplification mixture consisting of 50 ng genomic DNA, 0.20 µM of each primer, 10 mM Tris-HCl (pH 8), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of each dNTP, and 1 U of *Taq* DNA polymerase. DNA was amplified in five steps: 1) initial denaturation of the double strand at 94°C for 4 min; 2) denaturation at 94°C for 1 min; 3) annealing of primers at 62°C for 45 s; 4) extension at 72°C for 1 min; 5) a final extension at 72°C for 4 min. Steps 2, 3 and 4, corresponding to one cycle, were repeated 35 times.

Amplified fragments were digested in a reaction mixture containing 10 µL of the PCR product and 4 U of the restriction enzyme. Digestion mixtures were incubated in a thermocycler at 37°C for 4 hours. After digestion of the amplified products, DNA fragments were separated on 2% agarose gel in a horizontal electrophoresis system. A 100 bp molecular weight standard was applied to each gel to calculate the size of the amplified and digested fragments. DNA fragments were visualized on agarose gel by ethidium bromide staining and exposure to ultraviolet light.

Animals comprising *Bos taurus taurus* mtDNA were characterized by the presence of two restriction fragments of 406 and 349 bp and animals comprising *Bos taurus indicus* mtDNA presented only one undigested fragment of 755 bp (Figure 1). Only 2.26% (8/354) of the animals presented *Bos taurus indicus* mtDNA. These findings differ from those reported by Meirelles et al. (1999) who observed 21% (10/48) of individuals with *Bos taurus indicus* mtDNA for the ND5/*Hind*III polymorphism in a sample of Nellore purebred origin animals.

In spite of the differences, both studies confirmed major matrilineal *Bos taurus taurus* participation in American Zebu cattle formation. Mannen et al. (1998) found significant association between mtDNA polymorphisms and carcass traits in Japanese Black Cattle, specifically *longissimus* muscle area and beef marbling score, suggesting the existence of important cytoplasm genetic effects on production traits in beef cattle. Animals studied in the present work were originated from selected lineages for high productive ability (Brazilian Superyoung System: Unesp, FMVZ, Botucatu, SP), then the high frequency of *Bos taurus taurus* mtDNA in these animals can be a consequence of selection.

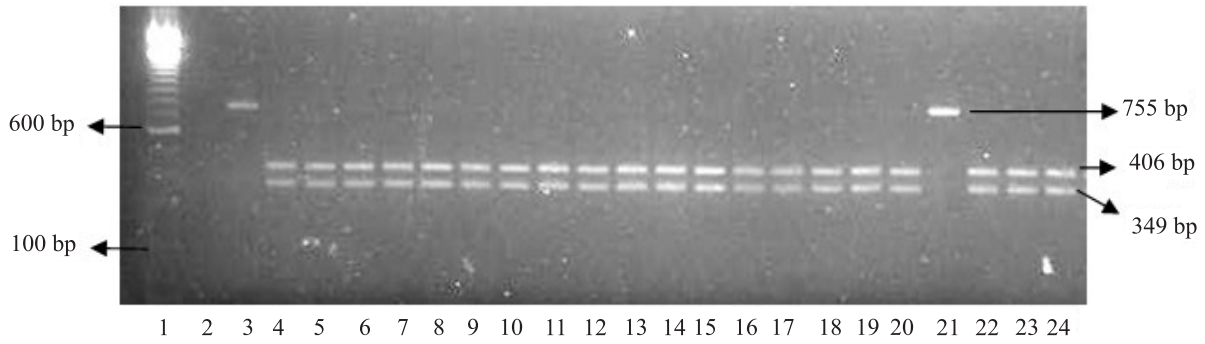

Figure 1. Ethidium bromide stained agarose gel electrophoresis of amplified fragment of ND5 gene of the bovine mtDNA digested with *Hind*III restriction enzyme. 1: DNA ladder 100 bp; 2: no DNA; 3: undigested DNA amplification; 4 to 20, 22, 23 and 24: *Bos taurus taurus* mtDNA pattern; 21: *Bos taurus indicus* mtDNA pattern. The numbers on the sides of the Figure indicate DNA fragments size.

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