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.

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 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 *HindIII* restriction enzyme (ND5/*HindIII* polymorphism).

The amplification of mtDNA was performed using
primers 5’– CCCACGAGGAAAATATACC – 3’
and 5’– GGAAGAGGTTTTGCGGTT – 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/*HindIII*
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.

![Ethidium bromide stained agarose gel electrophoresis of amplified fragment of ND5 gene of the bovine mtDNA digested with HindIII restriction enzyme.](image)

Figure 1. Ethidium bromide stained agarose gel electrophoresis of amplified fragment of ND5 gene of the bovine mtDNA digested with *HindIII* 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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References


