ABSTRACT: The present study aimed to analyze the association between copy number variation regions (CNVRs) and tenderness in 737 Nellore male cattle. The animals were finished in feedlot and slaughtered at approximately two years of age. Meat tenderness was analyzed in the Longissimus dorsi muscle. Samples with call rates below 90% were excluded. The PennCNV and CNVruler software were used to identify and link the CNVR with tenderness. The Log R Ratio (LRR) and B Allele Frequency (BAF) parameters were used to estimate the copy number variations (CNVs). A total of 4504 CNVRs were identified, with a mean length size of 36.59 kb. Only 10 CNVRs have MAF greater than 0.05%, where 2 CNVRs were significant associated (P<0.05) with meat tenderness. Meat tenderness must be improved in Zebu cattle, and CNVRs is a tool that can be used.

Key words: chromosomal region; CNVR; shear force.

INTRODUCTION
Tenderness is the main factor that influences meat’s organoleptic characteristics and its acceptance by consumers. It is known that meat from zebu breeds is not as tender as in taurine breeds due to lower carcass fat deposition, such as marbling, as well as the occurrence of higher levels of calpastatin in muscle. There are non-genetic methods (chemical and mechanical processes) that can induce or preserve meat tenderness. Genetic differences in marbling deposition and calpastatin activity, which are associated with meat tenderness, are exploited by the productive sector.

The copy number variation (CNV) is characterized by the loci breakpoint (initiation and termination sites), single copy length and copy number. Breakpoint and copy number estimations are always desirable (Duan et al. (2013)), referring to duplications or deletions of DNA segments larger than 1 kbp (Freeman et al. (2006)) and are important in order to explain the phenotypic variability of productive traits as well as susceptibility to disease. (Hou et al. (2012)), proposed to identify and characterize the CNVRs associated with feed efficiency during early lactation in Holstein females and found 443 significant genome regions, contributing to the differences in animal feed efficiency. Thus the present study aimed to identify association between CNVRs and meat tenderness in Nellore cattle.

MATERIAL AND METHODS
Animals and genotyping. A total of 737 Nellore bulls were finished in feedlot (minimum period of 90 days) and slaughtered at approximately two years of age. The carcasses were chilled for a period of 48 hours. During boning, a sample of one inch (2.54 cm) of Longissimus dorsi muscle was collected between the 12 and 13th ribs from the left carcass side. A Warner-Bratzler Shear device with 25 kilograms of force (kgf) ability and a speed of 20 cm/minute was used to determine tenderness. The shear force was the arithmetic mean of the cylinders, expressed in kgf. A panel with over 777,000 SNPs in the BovineSNP BeadChip (High-Density Bovine BeadChip) was used to genotype the animals.

CNV detection. The PennCNV algorithm (Wang et al. (2007)), which incorporates multiple information sources from genotyping data, was used for CNV detection. The parameters used for estimating the CNVs were: Log R Ratio (LRR) and B Allele Frequency (BAF). For quality control, samples with standard deviation values for LRR < 0.30, BAF derived as < 0.05 and waving factor < 0.01, were eliminated. The individual calls originated from the PennCNV software were grouped into CNVRs by the CNVRuler software (Kim et al. (2012)). The “recurrence 0.1” parameter (areas with low density are excluded, < 10% of CNVs, to compose an estimated end region) was used, which leaves a more robust delimitation of the beginning and end of regions. The “Gain/Loss separated regions” option was additionally used, which compiles the region based on the genotype (gain or loss of copy number) instead of composing regions ignoring the event type.

CNV association. A wide association analysis using adjusted phenotypic values was performed by applying a general linear model. The meat tenderness records were adjusted for the fixed effects of farm and year of birth, and management groups at birth, weaning and yearling. The model included the state of CNVR as a fixed effect and the age of animal at slaughter as a covariate (linear and quadratic effects). The CNVRs with Minor allele Frequency (MAF) less than 0.05 were excluded.

RESULTS AND DISCUSSION
A total of 4,504 CNVRs were identified in the autosomal chromosomes with an mean length size of 36.6 kb, of which, 54.71% presented base gain, 27.46% deletions and 17.81% mixed. Only 10 CNVRs had MAF greater than 0.05%, of which, only 2 were significant (P<0.05) when associated with meat tenderness (CNVR_1491 – p=0.02 and CNVR_1130 – p=0.05). The CNVR_2492 showed no significant p value (0.12), but is located in a region of interest (Table 1).

The CNVR_1491 (N=266), located on chromosome 7 in an intergenic region, possibly serving as an indirect genetic marker. This CNV is located between the LOC785149 and LOC100848897 regions. The first region...
is defined as not characterized and the second as a pseudogene for a transcription factor for heat shock proteins. Another nearby region, LOC100848873, 41 kb distant, is also a pseudogene for transcription factors for heat shock proteins. It is known that these heat shock proteins influence meat tenderness (Carvalho et al. (2014)) and therefore the significant p-value for the region is an indication that these genes may be influencing this trait. All these LOC regions cited above are "like" regions, ie areas that have partial or total similarity to genes in other genome regions.

The CNVR_1130 (N=406), located on chromosome 5 in an intergenic region since it is not proven to be on any gene. Within this CNV, four regions are noted yet, two are not characterized (LOC101903760 and LOC101903813). These two regions, when compared using the NCBI BLAST tool, do not point out any similar gene sequence. The other two regions (LOC101903866 and LOC101903350) are sequences "like" from the butyrophilin subfamily 1 member A1 gene. This protein is associated with the formation of fat droplets in (Muszyńska et al. (2010)). Probably, this gene may influence the subcutaneous fat and therefore affect meat tenderness. However, there is no evidence that these genes are being expressed muscle.

The CNVR_2492 (N = 291), located on chromosome 12, covers an uncharacterized sequence (LOC101904261). When compared to other available sequences, using the BLAST tool, indicates partial similarity to the ATP-binding cassette gene, sub-family C (CFTR/MPR), member 4. Among other functions, this gene controls the proliferation of smooth muscle cells. Although the skeletal muscle presents different structure and function to the smooth muscle, both have the same embryonic origin, characteristics and similar cells, being a possible reason of the relationship of this CNVR with meat tenderness. In particular, this region of chromosome 12 contains multiple, partial or complete, copies of the gene concerned. (Lee et al. (2013)) analyzing this gene in Hanwoo cattle, found a very large number of NS/SS/I (Non-synonymous SNPs, splice-site variants, and coding indels) reaching a hypothesis that this gene evolved into a gene with multiple copies for environment adaptation. Therefore, this CNVR may be the real cause of phenotype variation, possibly influencing changes in other nearby regions or being a combination of the two mentioned before, requiring a more detailed study to attempt to elucidate this query.

### Table 1. Identification, location and length size of CNVRs associated with meat tenderness.

<table>
<thead>
<tr>
<th>CNV ID</th>
<th>Chr</th>
<th>Start (kb)</th>
<th>End (kb)</th>
<th>Size (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNVR_1491</td>
<td>7</td>
<td>44437,3</td>
<td>44445</td>
<td>7,5</td>
</tr>
<tr>
<td>CNVR_1130</td>
<td>5</td>
<td>117282</td>
<td>117640</td>
<td>35,8</td>
</tr>
<tr>
<td>CNVR_2492</td>
<td>12</td>
<td>72748,5</td>
<td>72883</td>
<td>13,4</td>
</tr>
</tbody>
</table>

kb – kilo bases

### CONCLUSION

This study provides evidence for association between CNVRs on chromosomes 5, 7 and 12 with meat tenderness. Meat tenderness must be improved in Zebu cattle, and CNVRs are tools that can be used to improve this trait.

### LITERATURE CITED