ASSOCIATION BETWEEN SINGLE NUCLEOTIDE POLYMORPHISMS AND BACKFAT THICKNESS IN NELLORE CATTLE USING HIGH DENSITY PANELS

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Abstract – The objective of this work was to analyze the genomic association between SNP markers and backfat thickness in Nelore cattle, aiming to bring information for genetic evaluation of animals for this trait. A total of 740 Nellore males born in 2008 and 2009 were used. The contemporary groups were defined as: year, farm and management group at birth, and management group at weaning and yearling. The slaughter of animals occurred in commercial slaughterhouses and the measurement of backfat thickness was performed using calipers, measuring the fat layer at an angle of 45° from the geometric center of the sample, between the 12th and 13th ribs. The DNA was extracted from 5 grams of *Longissimus dorsi* muscle. The genotyping was performed using the high density panel, Illumina BovineHD BeadChip, with 777,962 SNP markers throughout the genome. After the quality control, 446,986 markers were available for genomic association. For backfat thickness, 68, 38 and 35 significant SNPs ($p<0.001$) were found, located respectively on chromosomes 7, 8 and 10.

Key Words – Beef cattle, Carcass traits, Genomic association, SNP markers

I. INTRODUCTION

In Brazil, genetic evaluation for *post mortem* carcass and meat traits, such as backfat thickness is not available in beef cattle breeding programs. Genome-wide association study (GWAS) is a new technique to identify causative genes for important traits in livestock. GWAS is used in existing variations (mainly single nucleotide polymorphisms, SNPs) throughout the genome, together with the phenotype and pedigree information, to perform association analyses and identify genes which are important for the traits of interest [1]. The molecular information obtained from these studies allows the genetic evaluation of young animals for economic traits and/or for difficult measurement traits [1]. In this study, we intended to analyze the genomic association between SNP markers and backfat thickness in Nellore cattle, aiming to bring subsidies for animals' genetic evaluation for this trait.

II. MATERIALS AND METHODS

A total of 740 Nellore males (not castrated) born in 2008 and 2009, progenies of 108 bulls, belonging to three breeding programs, were used. The contemporary groups (CG) were defined as: year, farm and management group at birth, and management group at weaning and yearling. A total of 117 CG was formed, with at least three animals in each CG.

The slaughter of animals occurred in commercial slaughterhouses and carcasses were chilled for at least 24 hours. After cooling, samples were taken from *Longissimus dorsi* muscle with bone (2.54 cm thick) between the 12th and 13th ribs of the left half carcass of each animal. The measurement of backfat thickness was performed using calipers, measuring the fat

layer at an angle of 45° from the geometric center of the sample, in millimeters.

For DNA extraction, 5 grams of *Longissimus dorsi* muscle was placed in a 2 ml eppendorf tube labeled with the animal number and stored in a freezer at -20 °C. The pieces of muscle tissue were weighed ranging from 25 to 30 milligrams in aluminum foil using an analytical balance and then placed in Eppendorf tubes (1.5 - 2 ml). DNA extraction was performed using the DNeasy Blood & Tissue Kit from Qiagen (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. The genotyping was performed at the Department of Technology (UNESP, Jaboticabal) using the high density panel Illumina BovineHD BeadChip with the HiScan™SQ System. The BovineHD BeadChip contains 777,962 SNP markers throughout the genome with an average distance between markers of 3.43 kb. The software GenomeStudio (Illumina®) was used to analyze the images of the HiScan and to obtain the genotypes.

The genomic data quality control and the preparation of files with genotypes were performed at the UNIX command prompt, present in Fedora operating system. The genotypes were defined as 0 (AA), 1 (AB) and 2 (BB). The criteria used for the quality control of genotyping data are shown in Table 1.

Table 1 Number of SNP markers excluded (N) for each criteria.

Exclusion criteria	N
MAF < 0.05	279,991
Heterozygosity > 0.30	851
Call Freq < 0.93	7,335
Average intensity of cluster > 0.30	12,630
Removal of the sex chromosomes	23,766
Removal of markers with A/B low frequency	6,403

The MAF (Minor Allele Frequency) represents the frequency of the less abundant allele, present in a given population. The Call Freq criterion is defined as the rate of assignment of genotypes, and represents the efficiency of genotyping for each SNP marker. In order to avoid possible errors of genotyping, SNPs with homozygotes and heterozygotes excess were excluded. The confidence intervals generated based on the population studied are called "clusters". SNPs away from these intervals were not considered. After the quality control, 446,986 markers remained for genomic association.

The association analyzes were performed considering only one marker at a time using the MIXED procedure of SAS software (version 9.2, SAS Institute Inc., NC, USA). The fixed effects in the model were: SNP marker, contemporary group, date of slaughter (15 levels) and slaughter age as covariate (linear effect). The false positive rate (FPR) was calculated as follows [2]: FPR = (m * p) / n, where *m* is the number of markers tested (markers that passed the quality control), *p* is the significance level and *n* is the number of significant markers (significance level < *p*) for backfat thickness.

III. RESULTS AND DISCUSSION

Applying Bonferroni correction for multiple tests ($p<1.12x10^{-7}$), no significant SNPs were found, probably because the correction applies a high level of restriction. According to [3] this is a conservative test. A total of 4,614 and 21,940 SNP markers associated significantly, $p<0.01$ and $p<0.05$, respectively, with the backfat thickness were observed. At $p<0.001$ a total of 653 SNPs were identified (Figure 1). The FPR, considering all significant SNPs ($p<0.001$), was 68%. This rate was lower than that found (84%) by [4] that performed an association study with 940 animals of different breeds for rump fat thickness. However, these authors used a panel with, approximately, 54,000 SNPs markers.

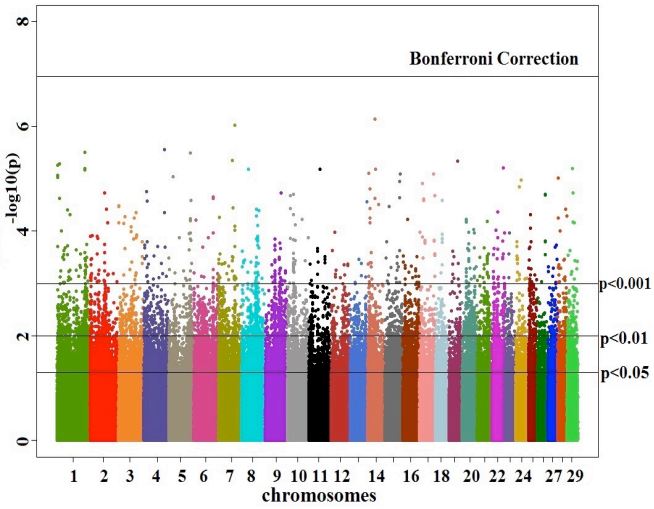

Figure 1. Manhattan plot for the genomic association with backfat thickness in Nellore cattle. Values above -logP>3 are equivalent to a p<0.001.

The distribution of SNPs in the manhattan plot shows larger peaks of associated markers (p<0.001) with backfat thickness in chromosomes 7, 8 and 10 (Figure 1) with 68, 38 and 35 significant SNPs, respectively. The chromosome 7 showed the highest number of significant SNPs (p<0.001). A total of 27 markers (p<0.001) were physically very close to each other (from 79,040 to 79,808 kb) in the chromosome 7. Using 312 markers in a population of 547 crossbred cattle (Brahman x Hereford), [5] reported significant markers for fat thickness on chromosomes 2, 3, 7 and 14. At chromosome 8, 7 markers (p<0.001) were in a region between 75,294 and 75,916 kb, i.e. physically very close. Similar results were reported by [6], who studied 89 markers in 455 animals from Belgian Blue, Piedmontese and Angus breeds, and found significant markers for backfat thickness (p<0.0003) on chromosome 8 and for intramuscular fat (p<0.0007) on chromosome 10. The results of the present study differ from those reported by [7], who identified significant SNP markers associated with backfat thickness at chromosomes 1, 5, 6, 19 and 21 in crossbred cattle (Angus x Charolais). Moreover, these results contrast with those reported by [8] that found 7 and 24 significant SNPs (p<0.001) for backfat thickness, at chromosomes 4 and 14, respectively. No QTL associated with backfat thickness in chromosome 10 is described in the Cattle QTL Database [9].

IV. CONCLUSION

SNPs markers significantly affecting backfat thickness at chromosomes 7, 8 and 10 were identified.

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