Genome-wide mapping of loci affecting pH in Nellore meat

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Abstract – The pH value is a key measurement of meat quality, since it influences various other parameters such as meat color, water holding capacity and tenderness. With the aim of mapping chromosome segments affecting pH in Nellore cattle, we carried out a genome-wide association study including 407 steers genotyped with the Illumina® BovineHD BeadChip assay. Resulting candidate regions and containing genes were investigated, but no previously described QTL, gene or promoter region directly related to biochemical processes responsible for pH variation were found. The functional enrichment analysis results showed no significant gene cluster potentially related to the trait. These results suggested that pH is mainly controlled by environmental factors, such as cattle handling during slaughter.

Key Words – GWAS, meat quality, SNP

I. INTRODUCTION

Usually, pH is the most commonly measured parameter in fresh meat, since it is related to biochemical processes involved in the muscle conversion to meat. Thus, its drop variation rate during the *post-mortem* period is related to the organoleptic meat characteristics. Generally, the final pH has a strong effect on meat color, water-holding capacity (WHC), flavor and tenderness [1]. Low pH levels adversely affect the functionality of the muscle proteins, reducing the color stability and water holding capacity, and may also influence the μ-calpain activity on autolysis, consequently altering proteolysis and meat tenderization [2]. The pH drop to lower levels than normal (5.5 – 5.8) during the *post-mortem*, causes meat darkening and reduces its tenderness, resulting in marketing depreciation [3].

Meat quality studies reported positive correlations between pH and shear force, including linear, quadratic and cubic pH effects on tenderness, with minimum values of objective tenderness observed at pH levels between 5.8 and 6.2 [4]. The activity of calpastatin, known inhibitor of calpain [5], is also positively influenced by higher pH values. The aforementioned relationships highlights the importance of this parameter on meat quality traits.

This study aimed at mapping chromosome segments affecting pH in Brazilian Nellore cattle (*Bos indicus*).

II. MATERIALS AND METHODS

A dataset of 407 Nellore steers genotyped with the Illumina® BovineHD BeadChip assay for more than 700 thousand single nucleotide polymorphism (SNP) markers was used. The pH phenotypic data was measured 24 hours after the slaughter by digital pHmeter. Only autosomal markers satisfying the following inclusion criteria were used in the downstream analyses: 1) minor allele frequency greater than 0.02, 2) Fisher's exact test P-value for Hardy-Weinberg Equilibrium greater than 1 x 10-20 and 3) call rate of at least 0.95. Only individuals with call rate greater than 0.9 were considered for analysis. The following mixed linear model was used for the association analysis: pH24 ~ mean + age + batch + SNP + animal + error. Animal and error were modeled as random effects, assuming multivariate normal distributions. Errors were assumed independently and identically distributed, whereas the covariance in animal effects was modeled using an additive relationship matrix computed from marker genotypes. All remaining variables were fitted as fixed effects. Markers presenting p value < 10^{-4} were prioritized for investigation. These analyses were performed using SNP and Variation Suite (SVS – Golden Helix).

The chromosomal regions containing significant association between trait and SNP were explored using the Ensembl release 75 BioMart data-mining tool [6] to export a custom dataset containing all genes inside a 1Mb window centered in the most significant marker position. Furthermore, in order to extract biological meaning out of the obtained gene list, data were analyzed using DAVID v6.7 functional annotation tools [7][8]. Functional enrichment analyses were performed in a stepwise manner to highlight the most relevant annotation terms associated to this gene list.

III. RESULTS AND DISCUSSION

After data filtering, a total of 513,724 SNP were included in the association analyses. Inflation factor was close to 1 ($\lambda = 0.9713$), indicating that no important confounding effects were neglected. Figure 1 shows the Manhattan plot and P-P plot of the association results.

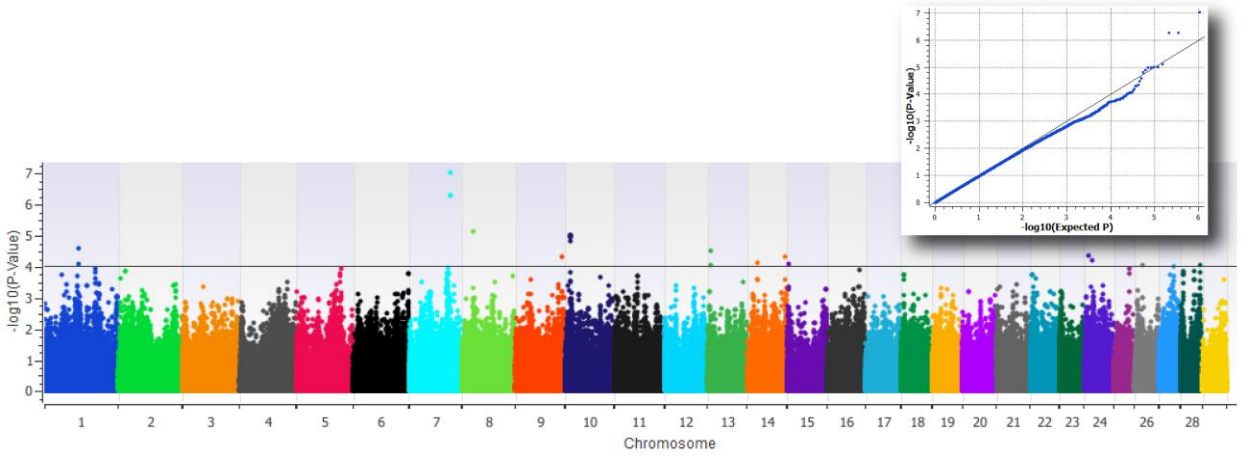

Figure 1. Manhattan plot of genome-wide -log10(p-values) for pH 24 hours in Nellore cattle, where the horizontal solid line represents the significance level adopted (α = 1x10-4); Percentile-percentile plot for expected and observed-log10(p-values).

Only SNPs above significance threshold were considered to be associated with the trait. Hence, putative associations were found in 13 regions through the genome, on chromosomes: BTA1, BTA 7, BTA8, BTA9, BTA10, BTA13, BTA14, BTA15, BTA24, BTA26, BTA27, BTA27 and BTA18. Inside those regions were identified 86 protein-coding genes, miRNA, misc_RNA, processed pseudogenes, pseudogenes, rRNA, snoRNA and snRNA.

The most significant SNP was located on BTA7:87663044pb, which differs from results found in other genome association study for the same cattle breed and trait. In that study, the major associated region was reported at 87Mb in chromosome 8 [9], while in our study the same chromosome showed associations at 24,6Mb.

Only known or known by projection genes were submitted to enrichment analysis on David v6.7, totaling 65 genes, but no significant cluster was found.

The QTLs query from the overlap of associated regions and the QTLdb database [10] did not revealed any previously reported QTL that could explain the pH variation or even metabolic pathways in close relation to this trait, which suggests a complex and small contribution of genetic variation to the regulation of the trait.

Several studies showed that pre-slaughter stress, fasting duration and water diet, hormonal changes and temperature elevation immediately before slaughter, causes pH abnormal variation due to partial or total depletion of glycogen storage in the muscle of these animals [3,11]. This reinforces how pre-slaughter factors influence the meat final pH value.

In addition to the known "pre" and "during" slaughter handling effects on meat quality, more

than 30 genes are known to be involved in the synthesis and degradation of glycogen and glucose, energy sources to the muscle [12], responsible for the pH variation in *post-mortem* muscle. Thus, a validation study with existing markers in these genes can enhance the results of genomic association studies.

IV. CONCLUSION

Lack of links between pH values and its genetic counterpart could indicate that this trait is mainly controlled by environmental factors. In order to confirm these results we will increase the number of evaluated animals to improve association significance levels and refine obtained regions.

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