CATTLE TEMPERAMENT AND *LONGISSIMUS* MUSCLE METABOLITES

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I. INTRODUCTION

Modifications in the rate of pH decline is the result of muscle energy *status* in the early *post mortem* period. In the case of *Longissimus* muscle, which is mostly composed of type IIx fibers, greater mitochondrial function and ability to maintain ATP levels during early *post mortem* is associated with delayed pH decline [1]. Animal responsiveness to stress pre-slaughter can affect the muscular ATP balance. Considering that beef from excitable animals has been linked to inferior quality [2], it is important to investigate mechanisms behind the stress physiology in animals with divergent temperaments. In this context, metabolomics is a powerful tool to unravel pathways that are associated with temperament, providing information that can contribute to easily and early access beef with superior quality. Therefore, our objective was to compare *Longissimus* metabolites in the early *post mortem* (1h) from calm and excitable Nellore cattle.

II. MATERIALS AND METHODS

All experimental procedures involving animal care were conducted in accordance with the Institutional Animal Care and Use Committee Guidelines of the College of Animal Science and Food Engineering at the University of Sao Paulo (6493190121). From a larger group of 72 Nellore males, a sub-group (n = 22) was selected based on temperament tests during the first handling (after animals were transferred from pasture and adapted to feedlot). Chute score and flight speed were determined and averaged to calculate temperament index. The index was used to classify animals either as excitable or calm. Care was taken to select progenies from several bulls in each group. Approximately 1h after slaughter, a small sample was taken from each carcass from the *Longissimus* muscle and between the 12th and 13th ribs, immediately frozen using liquid nitrogen and stored in an ultra-freezer (-80 °C) until processing. Metabolites were extracted using methanol/chloroform/water (2/2/1, v/v/v), as previously described by [3] and analyzed through nuclear magnetic resonance spectrometry (^{1}H-NMR). 1D ^{1}H-NMR spectra were processed, metabolites were identified and quantified using the Chenomx NMR Suite Professional 10.0 software (Chenomx Inc., Edmonton, Canada). Metabolomic data were analyzed using MetaboAnalyst 6.0 (http://www.metaboanalyst.ca/), through Volcano Plot and enrichment analysis.

III. RESULTS AND DISCUSSION

Based on the Volcano Plot, the metabolites that explained most of the data variability were isovalerate, adenine, taurine, glucose, sarcosine, acetate, o-acetylcarnitine and glutathione (Figure 1). The overview of enriched metabolite sets (Table 1) showed that the ‘taurine and hypotaurine metabolism’ was the most significant pathway that helped to differentiate metabolites from the divergent temperaments. This pathway is mostly explained by taurine abundance, which is greater (P = 0.02) in muscle from excitable animals. The second most significant pathway was the ‘glucose-alanine cycle’,

which was mostly explained by glucose and glutamic acid abundance. In this case, d-glucose was greater (P = 0.02) in muscle from excitable animals, while glutamic acid did not differ between groups. The third most significant pathway was the 'transfer of acetyl groups into mitochondria', and the most important metabolites involved were d-glucose, malic acid and adenosine triphosphate (ATP). In this case, the relative abundance of the malic acid and ATP did not differ between groups.

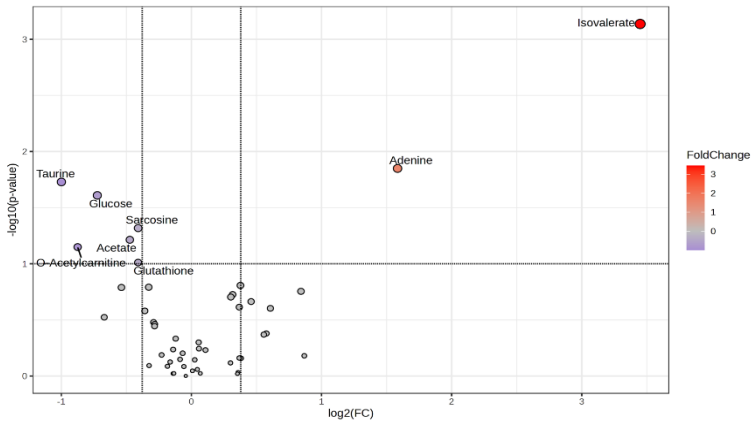

Figure 1. Volcano plot of metabolites from *Longissimus* muscle 1h *post mortem* from Nellore cattle with calm and excitable temperaments

Table 1 – Overview of enriched metabolites sets (top 4) from *Longissimus* muscle 1h *post mortem* from Nellore cattle with calm and excitable temperaments[1].

Pathways	P value	Abundant metabolites
Taurine and hypotaurine metabolism	0.018	Taurine
Glucose-alanine cycle	0.032	D-glucose and glutamic acid
Transfer of acetyl groups into mitochondria	0.046	D-glucose, malic acid, and ATP
Sphingolipid metabolism	0.047	D-glucose and ATP

[1]Temperaments groups were selected based on the temperament index calculated from chute score and flight speed average

IV. CONCLUSION

Nellore cattle classified as excitable have a specific metabolite profile within *Longissimus* muscle at 1h *post mortem* that differs from calm animals. Further research is needed to elucidate the relationship between the profile and early *post mortem* metabolism in bovine with divergent temperaments.

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