Metabolomic approach for determination of compounds and pathways related to beef quality

Mariane Beline [1], Daniel S. Antonelo [1], Juan F. M. Gomez [1], Luiz A. Colnago [3], Angel R. H. Padilla [3], Alessandra F. Rosa [1], Mirele D. Poleti [1], Julio C. C. Balieiro [1], David E. Gerrard [2], Saulo L. Silva [1]

[1] University of Sao Paulo, [2] Virginia Tech, [3] EMBRAPA, Brazil

Objective: The aim of this study was to use a non-targeted metabolomic approach to explain differences in meat quality traits across gender status.

Materials and Methods: Eighty Nellore cattle were used in a completely randomized design with two treatments, castrated (C; n=40) and non-castrated (NC; n=40). Surgical castration was performed at weaning (8-10 mo.old). Animals were raised on grass until 20 mo of age, then finished in feedlot for approximately 88 days. Twenty-four hours after harvest, muscle pH was measured and approximately 10g *Longissimus thoracis* (LT) muscle, opposite the 12th rib, was sampled for determination of glycogen con- centration, metabolomic analysis, and one 2.5 cm thick steak was collected for meat color and tenderness analyses.

Results and Discussion: Glycogen concentration was greater in muscle from C cattle (18.5 versus 7.6μmol/g; P=0.030), while the ultimate pH was higher in NC muscle (5.73 versus 5.60; P<0.001). Meat from NC cattle had lower L* (37.4 versus 39.1; P=0.040), a* (14.6 versus 16.2; P=0.001) and b* values (12.0 versus 13.8; P=0.002) compared to that of C cattle. Meat from NC cattle was less tender (12.0 versus 9.9kg; P=0.005) than that of C cattle. The 5 most important metabolites that explained residuals in the PLS-DA plot were creatine, pyruvate, glycine, choline, and betaine, respectively. Meat from C cattle presented with greater concentrations of betaine (P=0.024), choline (P=0.045), pyruvate (P<0.001), and xanthine (P=0.002), and lower concentrations of creatine (P=0.002) than meat from NC cattle. The most relevant pathways associated with the identified metabolites were glycine, ser- ine, and threonine, pyruvate, glycolysis/gluconeogenesis, and citrate cycle. More tender and brighter colored lean was observed in C cattle. Rate and extent of pH decline are two of the most important factors altering meat quality because it influences meat color and tenderness. Meat from NC cattle had higher ultimate pH values, which is generally associated with DFD meat because lower glycogen concentrations are caused by pre-slaughter stress. However, while glycogen concentrations in LT were indeed lower in muscle from NC cattle, levels were sufficient to reach normal pH values by 24hr postmortem in both treatments. Furthermore, dif- ferences in pH, tenderness and color still existed in the presence of significant residual glycogen, arguing that other factors may be responsible for differences in these highly coveted fresh meat quality attributes aside from resting glycogen content and(or) glycolytic flux. Even so and based on metabolome data, carbohydrate metabolism remained as the most relevant pathway associated with the metabolites identified in this study. Specifically, the TCA cycle and glycolysis/gluconeogenesis were the most significant pathways explaining differences in meat across gender, namely those pathways responsible for maintaining ATP homeostasis in the tissue. Meat from NC cattle had higher concentrations of creatine, which has a positive effect on mitochondria function and structure, and easily explain the reduced amounts of pyruvate in muscle of NC because pyruvate would be rapidly oxidized in mitochondria via the TCA cycle (Zangari et al., 2020). According to Picard et al. (2019), a greater percentage of slow-twitch oxidative fibers are observed in muscle of bulls, and along with higher creatine levels, favor mitochondria function and pyruvate oxidation. Higher betaine and pyruvate in C muscle suggest higher antioxidant capacity compared to that of NC cattle (Zhang et al., 2016). Increased muscle antioxidant capacity increases color stability (Ramanathan et al., 2012) and raw beef color (Suman et al., 2014), corroborating more favorable color values in meat from steers. Xanthine production, which was higher in NC cattle muscle, is as- sociated with ROS production and results in oxidative stress, thus inactivating μ-calpain and consequently reducing proteolysis in beef (Lametsch et al., 2008) which agrees with the lower tenderness observed in meat from bulls in this study.

Conclusion: In summary, beef quality traits and metabolites are affected by gender status. Metabolite profiles reveal that muscle from steers is more glycolytic and possesses more metabolites with antioxidant properties, and these characteristics may influence meat quality development compared to that of muscle from bulls.

References:

Lametsch, R.; Lonergan, S.; Huff-Lonergan, E. Disulfide Bond within μ-Calpain Active Site Inhibits Activity and Autolysis. Biochi- mica et Biophysica Acta 2008, doi:10.1016/j.bbapap.2008.04.018.

Picard, B.; Gagaoua, M.; al Jammas, M.; Bonnet, M. Beef Tenderness and Intramuscular Fat Proteomic Biomarkers: Effect of Gender and Rearing Practices. Journal of Proteomics 2019, doi:10.1016/j.jprot.2019.03.010.

Ramanathan, R.; Mancini, R.A.; van Buiten, C.B.; Suman, S.P.; Beach, C.M. Effects of Pyruvate on Lipid Oxidation and Ground Beef Color. Journal of Food Science 2012, doi:10.1111/j.1750-3841.2012.02814.x.

Suman, S.P.; Hunt, M.C.; Nair, M.N.; Rentfrow, G. Improving Beef Color Stability: Practical Strategies and Underlying Mechanisms. Meat Science 2014, doi:10.1016/j.meatsci.2014.06.032.

Zangari, J.; Petrelli, F.; Maillot, B.; Martinou, J.C. The Multifaceted Pyruvate Metabolism: Role of the Mitochondrial Pyruvate Carri- er. Biomolecules 2020, doi:10.3390/biom10071068.

Zhang, M.; Zhang, H.; Li, H.; Lai, F.; Li, X.; Tang, Y.; Min, T.; Wu, H. Antioxidant Mechanism of Betaine without Free Radical Scavenging Ability. Journal of Agricultural and Food Chemistry 2016,

doi:10.1021/acs.jafc.6b03592.

Key words: Castration, Gender, Meat color, Meat tenderness, Metabolome