

Protein profile in longissimus thoracis muscle from zebu cattle reveals a protein that may be related to intramuscular collagen solubility

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Introduction: The intramuscular collagen solubility (ICS) differs among breeds through variation in animal physiological maturity and also due to differences in precocity between distinct muscles and breeds (BLANCO et al., 2013). The goal of this study is to evaluate the effects of contrasting Expected Progeny Differences (EPDs) for precocity and growth of Nelore cattle by differential proteomics and intramuscular collagen solubility analysis on *longissimus thoracis* muscle (LT).

Material and Methods: One hundred and five bulls from the same herd and knew genetic information of precocity and growth were used, with a mean age of 20 ± 2 months and 400 ± 24 kg. The bulls were kept in the feedlot, fed the same diet for 100 days, and subsequently slaughtered. Samples of LT were collected at the slaughter for proteomic analysis. After a 24h chilling period, steaks were cut from LT for ICSA. The animals were selected according to their fathers' EPDs. Six fathers with EPDs contrasting simultaneously for precocity and growth were selected so that each experimental group had three different fathers. Then, to evaluate ICS two contrasting groups were formed, called high EPD (H_EPD; N = 16) and low EPD (L_EPD; N = 16). Posteriorly, 14 animals were selected (H_EPD; N = 7 and L_EPD; N = 7) to form the groups that were tested for differential proteomic analysis. In statistical terms to detect the differences between the abundance of each band between groups ($P < 0.1$) was used Tukey-Kramer test revealed by one-dimensional electrophoresis (DÍAZ et al., 2020). The correlations between the bands and the phenotypes were evaluated by Pearson's correlation coefficient (r), within and between groups ($P < 0.1$). The differentially abundant bands were cut from the gel and submitted to LC-MS/MS. The identified proteins were considered candidates when the global Mascot score was greater than 57 ($P \leq 0.0001$).

Results: The H_EPD group showed greater ICS than the L_EPD group ($P = 0.098$). The 16 electrophoretic band was differentially abundant ($P = 0.013$) between the groups H_EPD (1.3872 ± 0.079) and L_EPD (1.9821 ± 0.079). We observed changes in the abundance of proteins between the contrasting groups, such as GPI, PKM, CASQ1, DLD, VIM, PKLR, PCYOX1, ALDOA, OXCT1, and ACTC1. It was observed a positive correlation between the 16 band and TGFB1 gene within the H_EPD group ($r = 0.91$; $P = 0.08$) and independent of the group ($r = 0.72$; $P < 0.05$). Therefore, the H_EPD group may have higher collagen turnover due to their higher growth rate and consequently can present higher solubility due to the lower protein abundance, especially the vimentin protein (VIM). The VIM is a protein that functions within the positive regulation of the intramuscular collagen biosynthetic process and is physiologically related to SMAD proteins and the TGFB1 gene involved in fibrogenesis (UniProt, 2021).

Conclusions: In conclusion, the EPD contrasting for precocity and growth of Nelore cattle influences muscle proteomics and intramuscular collagen solubility. Furthermore, the VIM protein can be associated with intramuscular collagen solubility in Zebu cattle selected for growth and precocity.

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Literature:

Blanco, M.; Jurie, C.; Micol, D.; Agabriel, J.; Picard, B.; Garcia-Launay, F. Impact of animal and management factors on collagen characteristics in beef: A meta-analysis approach. *Animal*, v. 7, p. 1208-1218, 2013.

Díaz, F.; Díaz-Luis A.; Sierra, V.; Diñeiro, Y.; González, P.; García-Torres, S.; Tejerina, D.; Romero-Fernández, M. P.; Cabeza de Vaca, M.; Coto-Montes, A.; Oliván, M. What functional proteomic and biochemical analysis tell us about animal stress in beef? *Journal of Proteomics*, v. 218, p. 103722. 2020.

The UniProt Consortium, UniProt: the universal protein knowledgebase in 2021, *Nucleic Acids Research*, v. 49 (1), p. D480-D489, 2021.