DIFFERENTIAL BEEF PROTEOMIC ANALYSIS BETWEEN STEERS AND BULLS

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I. OBJECTIVES

The study aims to identify differentially abundant proteins between 2 sexual conditions (steers and bulls) in Nelore cattle and to associate them with beef quality traits and metabolites.

II. MATERIALS AND METHODS

The analysis of beef quality (color, pH, cooking losses, Warner-Bratzler shear force at 1, 7, and 14 d postmortem) and muscle metabolites (lactate and glycogen at 1 and 24 h postmortem) were carried out in *Longissimus thoracis* samples from a population of 200 Nelore cattle, 100 steers (castrated), and 100 bulls (noncastrated). The animals were finished in the feedlot and slaughtered at 24 mo old and live weight 505 kg. The carcasses were chilled, and *Longissimus thoracis* muscle samples were excised at 24 h after slaughter. We used samples from 6 animals of each sexual condition ($N = 12$) chosen randomly to carried out the proteomic analysis. Total proteins were extracted by homogenizing these samples in lysis buffer (8 M urea, 2 M thiourea, 1% DTT, 2% CHAPS, and 1% protease inhibitor cocktail), vortexing, and centrifuging. The protein concentrations of the supernatant were determined. The proteins were digested by trypsin, and the mass spectra of the peptide fragments were acquired by the bidimensional nanoUPLC tandem nanoESI-HDMS^E technology system. Mass spectrometry data were processed using ProteinLynx Global Server version 2.5.1 (PLGS, Waters Corporation) software platform. False positive discovery rate of the identification algorithm was set to 4%. For statistical analysis, only proteins present in at least 3 of the 6 biological repetitions were considered ($N = 198$) to detect differentially abundant proteins. Variance analyses (analysis of variance) were performed using a mixed linear model, including the fixed effect of sexual condition and origin and slaughter data as random effects. Correlations were estimated by Pearson's correlation coefficient (r) and considered significant at $P < 0.05$.

III. RESULTS

A total of 605 proteins were identified. Of these, 330 were common between groups, 139 were found only in steers, and 136 were found only in bulls. However, we considered potential proteins exclusive for each sexual condition when they were found in 5 of the 6 biological replicates. Therefore, 5 proteins (CX6A2, ANXA2, F1MS25, F1N206, PROF1) were considered exclusive for the steers, and 2 proteins (MYH3 and ACBP) were deemed to be unique to the bulls. Nine proteins were differentially abundant ($P < 0.05$) between sexual conditions. Three proteins play a structural role (MYBPC1, MYH4, MYL6B), 5 are metabolic pathway proteins (AGL, PYGM, PRDX6, ACO2, HADHA), and 1 is involved in the epigenetic regulation of gene expression (APOBEC2). APOBEC2, AGL, PRDX6, and ACO2 showed positive correlations ($r > 0.65$) with color values (L^*, a^*, b^*), and the last 2 proteins had a negative correlation with Warner-Bratzler shear force at 14 d of aging ($r = -0.64$ and $r = -0.70$,

respectively). Lactate measured 1 h postmortem was negatively correlated with MYH4 ($r = -0.68$), and lactate measured 24 h postmortem was positively correlated with HADHA abundance ($r = 0.68$).

IV. CONCLUSION

Overall, our findings demonstrated that (a) sexual condition modified the beef proteome and (b) meat quality and muscle metabolite traits were differentially associated with specific proteins for steers and bulls.

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