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STRUCTURAL PROTEINS AS BIOLOGICAL MARKERS ASSOCIATED WITH MEAT TENDERNESS IN NELLORE CATTLE

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Abstract: O presente trabalho descreve a associação da maciez da carne com a expressão de proteínas do músculo esquelético de bovinos da raça Nelore. Três grupos experimentais de carne moderadamente macia, moderadamente dura e muito dura foram selecionados por meio das analises de força de cisalhamento e índice de fragmentação miofibrilar. A análise proteômica foi realizada utilizando a técnica de 2D-PAGE e espectrometria de massas. A expressão das proteínas estruturais α-actina (ACTA1), β-actina (ACTB), γ-actina (ACTG1) e isoforma da cadeia leve de miosina 1/3 (MYL1) foram *down-regulated* ($p<0.05$) no grupo de carne moderadamente macia, quando comparado com os demais grupos. A profilina 1 (PFN1) foi *up-regulated* ($p<0.05$) no grupo de carne moderadamente macia. Os resultados demonstram que o amaciamento da carne em bovinos Nelore depende da modulação e expressão das proteínas estruturais e sugere que estas proteínas possam ser marcadores biológicos associados com a maciez da carne de bovinos.

Keywords: beef cattle, shear force, 2D-PAGE

Introduction

Tenderness is considered the most important meat quality trait and proteins play a fundamental role in the regulation of the metabolic changes required for the

conversion of muscle to meat. Within this context, recent studies have used tools such as proteomics to understand the biochemical engineering involved in the process of protein denaturation during the postmortem period in Nellore cattle (Baldassini et al., 2015; Rosa et al., 2018).

Meat tenderization in cattle is a complex process and is specific for each breed, further studies that investigate different tenderness groups using adequate biotechnological tools and analysis methods are needed to increase our understanding of the biochemical engineering involved in the postmortem meat tenderization process in Nellore cattle. Within this context, the objective of the present study was to investigate and evaluate the proteomic profile of *Longissimus thoracis* muscle in different meat tenderness groups of Nellore cattle.

Material and Methods

A population of 90 contemporaneous uncastrated, male Nellore animals with mean initial weight of 390 ± 37 kg was used. The finishing period in the Experimental Feedlot of Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Unesp, Botucatu, SP, Brazil, lasted 95 days. The procedures were approved by the Ethics Committee on Animal Experimentation of FMVZ, Unesp, Botucatu/SP (Protocol No. 159/2014). The animals were randomly allocated to 18 collective and received the same diet. Next, the animals with final weight of approximately 550 ± 75 kg and 27 months of age, they were slaughtered. After slaughter, the carcasses were cooled for 24 h. Next, *Longissimus thoracis* muscle samples were collected between the 12th and 13th rib of each animal for analysis of shear force (SF), myofibrillar fragmentation index (MFI) and for two-dimensional polyacrylamide gel electrophoresis (2D-PAGE).

The meat samples were aged for two different periods of time (24 h and 7 days after slaughter) for SF and MFI analysis performed according to the methods of Wheeler et al. (1995) and Chardulo et al. (2014), respectively. Next, three contrasting meat tenderness groups were selected: moderately tender meat (SF24 =

3.9 ± 0.7 kg; SF7 = 3.4 ± 0.4 kg; MFI24 = 55.7 ± 9.0; MFI7 = 65.9 ± 7.3; n = 15), moderately tough meat (SF24 = 5.6 ± 0.7 kg; SF7 = 4.5 ± 0.4 kg; MFI24 = 50.4 ± 13.9; MFI7 = 58.2 ± 13.2; n = 20), and very tough meat (SF24 = 7.9 ± 1.4 kg; SF7 = 5.9 ± 0.6 kg; MFI24 = 40.1 ± 8.9; MFI7 = 44.8 ± 4.8; n = 20). After division of the groups, SF and MFI were submitted to multivariate statistics by principal component analysis and the groups were divided into subgroups with 5 animals/subgroup/pool.

The proteins were extracted of each subgroup and applied to 13 cm strips with ampholytes immobilized at pH 3-10. Electrophoresis in the first dimension was performed in an Ettan™ IPGphor™ 3 system (GE Healthcare). For the second dimension, the strips were placed on polyacrylamide gel (12.5%, w/v), together with a molecular weight standard (14.4 - 97.0 kDa). The gels were scanned with ImageScanner III (GE Healthcare) and the images were analyzed with the ImageMaster Platinum7.0 program (GE Healthcare). The protein spots differentially expressed were selected, and analyzed in the nanoACQUITY UPLC-Xevo TQ-MS System (Waters, Manchester, UK). The proteins were identified by UniProt database (UniProtKB/Swiss-Prot, www.uniprot.org) for the *Bos taurus* genome.

Results and Discussion

Pairwise comparison of the gels was used because image treatment is a time-consuming process. After the identification of matching spots, the correlation between the pairs of gels within each subgroup was calculated. The average correlation between the gel replicates was 96%, 91% and 93% for the moderately tender, moderately tough and very tough meat groups, respectively, and the mean number of spots was 216.0 ± 11.6, 222 ± 12.9 and 224 ± 10.4. The correlations between protein spots were estimated by image analysis considering the percentage of spot volume normalized during the matching process. The correlations between protein spots of the moderately tender and moderately tough meat groups (R>0.82), moderately tender and very tough meat groups (R>0.76), and moderately tough and

very tough meat groups (R>0.89) indicated the proximity of the proteomic profiles of the groups.

The distribution of the diversity of protein spots in the three experimental groups was homogenous, with a Mw ranging from 20.1 to 66 kDa and pI ranging from 5 to 7. These results correspond to the protein fraction obtained by Bjarnadóttir et al. (2012) for *Longissimus dorsi* muscle of young Norwegian Red cattle and by Baldassini et al.(2015) for *Longissimus thoracis* of Nellore cattle.

The greatest differences in expression ($p<0.05$) were found for the structural proteins α-actin (ACTA1), β-actin (ACTB), γ-actin (ACTG1), myosin light chain 1/3, skeletal muscle isoform (MYL1), and profilin 1 (PFN1). The spots of the ACTA1, ACTB, ACTG1, and MYL1 were significantly down-regulated ($p<0.05$) in the moderately tender and moderately tough meat groups compared to the very tough meat group. These proteins have usually been associated with meat tenderness in cattle. For example, α-actin has been suggested as an early and adequate predictor of postmortem proteolysis in Norwegian Red cattle (Bjarnadóttir et al., 2012) and was found to be associated with meat tenderness in Nellore cattle (Rosa et al., 2018). Similarly, as observed in the present study, higher expression of MYL1 has been demonstrated in meat with lower tenderness (Rosa et al., 2018).

On the other hand, structural protein profilin 1 (PFN1) was up-regulated ($p<0.05$) in the moderately tender meat group compared to the other groups. PFN1 has not been investigated in studies on meat tenderness. According to the literature, in mammals PFN1 binds to actin and accelerates the exchange of ADP for ATP, thus affecting the structure of the cytoskeleton (Courtemanche & Pollard, 2013). The postmortem regulation of ATP is directly related to the formation of the actin-myosin complex, which is one of the main components responsible for the conversion of muscle to meat. Thus, the positive association of PFN1 with meat tenderness observed in this study may be due to the action of this protein on actin. However, further studies are necessary to confirm this hypothesis.

Conclusion

The results of the present study suggest of structural proteins as possible biological markers associated with meat tenderness in Nellore cattle.

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