PROTEOME CHANGES OF BEEF IN NELLORE CATTLE (*BOS INDICUS*) WITH DIFFERENT GENOTYPES FOR TENDERNESS

Alessandra F. Rosa[1]; Joanir P. Eler.[1]; Mirele D. Poleti[1]; Cristina T. Moncau[1]; Vanessa A.M. Silva[1]; Saulo L. e Silva[1]; Fabio M. Moraes[1]; Marco A. Trindade[1]; Julio C.C. Balieiro[1]

College of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil;

[2]Department of Food Engineering, College of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil

Abstract – The aim of this study was to evaluate changes in the protein profile of Nellore cattle beef with different genotypes for tenderness, using two-dimensional (2DE) gel electrophoresis. For this purpose, 155 animals (80 steers and 75 young bulls, 23-month old) from the beef cattle herd of University of São Paulo were feedlot finished and then slaughtered. Cattle were genotyped for a single nucleotide polymorphism (SNP) in the calpain (CAPN) and calpastatin (UOGCAST) genes. The 2DE was carried out in LM samples collected 24 h post mortem for each genotype group. The genotypic frequencies for CAPN were TT=69.06%; CT=28.4% and CC=2.6% and for UOGCAST were GG=13.6%; CG=51.6% and CC=34.8%. The 2DE analysis shows, 174 spots in common for all gels. From those, 41 spots changed significantly (P<0.05). There are some differences in the protein profile of different genotypes, but more studies are necessary to elucidate how these differences affect meat tenderness.

Key Words – 2DE electrophoresis, meat quality, molecular marker

I. INTRODUCTION

Solving the problem of inconsistent meat tenderness is a top priority of the meat industry. This requires a greater understanding of the processes that affect meat tenderness and, perhaps more importantly, the adoption of such information by the meat industry [1].

Genetic improvement has long been considered an important factor in the competitiveness of beef cattle production for enhancing product quality. Identification of the genes and/or polymorphisms underlying quantitative/qualitative traits, and an understanding of how these genes/polymorphisms interact with the environment or with other genes affecting economic traits might be the keys to successful application of marker-assisted selection in the commercial animal population [2]. Although the *post-mortem* degradation of a series of structural proteins have been studied extensively in recent years has not been possible to establish whether this fact in itself, is directly responsible for the tenderization of the meat. This is explained by the fact that the resolving power of low-dimensional electrophoresis has been the most used technique in these studies. Proteomic analysis of gels based on two-dimensional electrophoresis and mass spectrometry are much more informative than the one-dimensional electrophoresis [3].

Thus, the aim of this study was to evaluate changes in the protein profile of Nellore cattle beef with different genotypes for tenderness, using two-dimensional (2DE) gel electrophoresis.

II. MATERIALS AND METHODS

A. Animals and Experimental Procedure

The research was conducted in the College of Animal Science and Food Engineering (FZEA) at the University of São Paulo (USP), Brazil. Throughout 2009 and 2010, Nellore bulls (n=75, 523 ±3.7 BW, 23-mo old) and steers (n=80, 483 ±32.4 BW, 23-mo old) were finished in feedlots receiving the same high-grain diets for all period (140 days).

Animals were slaughtered according to standard humane procedures at a local slaughterhouse. The captive bolt method was used to stun the animals. Carcasses were split, weighed and then chilled at 0-3ºC before processing on the following day after slaughter.

B. Marker Used

Cattle were genotyped for a single nucleotide polymorphism (SNP) in the calpain (CAPN4751; GeneBank accession number: AF248054, position 6545) and calpastatin genes (UOGCAST; GeneBank accession number: AY008267, position 282).

C. Genotyping

Blood samples were collected and DNA prepared from these animals was storage -80ºC. The CAPN and UOGCAST SNPs were genotyped by Real Time PCR (ABI Prism® 7500 Sequence Detection System – Applied Biosystem). The PCR master mix was 0.25µl Assay Mix® (Applied Biosystem), 5µl Taqman® Master Mix Universal PCR (Applied Biosystem), and 15 ng of DNA for 10µl total volume.

The PCR cycling condition was 95ºC (10.0 min) for 1 cycle; then 92ºC (15 s) for 45 cycles and 1 to 60ºC (60s) maintaining 4oC thereafter.

D. Proteomic analysis

Muscle Samples

The 2DE was carried out in *Longissimus Dorsi* muscle samples (1g) collected 24 h *post mortem* for each genotype group.

Six samples of 1g muscle tissue of each genotype group were mixed in glass plate. This mix 0,5g was collected placed in a Falcon Tube and homogenized in 5 mL of 8 M Urea, 2 M Thiourea, 65 mM DTT, 2% CHAPS [10] using a Turratec homogenizer for 60 seconds at 16000 r.p.m.. Crude extracts were transferred to Erlenmeyer's flaks, vigorously shaken for 2 hours, and centrifuged (30 min. at 10 000 x g) in order to remove unextracted cellular components, high molecular weight protein complexes, and insoluble proteins [7]. The protein content was determined with 2-D Quant Kit (GE Healthcare) The protein extract obtained was placed in microtubes and stored at -80°C.

Two-Dimensional Electrophoresis (2-DE)

To perform the 2DE Strips IPG pH 4-7 (GE Healthcare), 13cm length, were used. The isoelectric focusing was performed with Ettan IPGphor (GE Healthcare) for separation of proteins according to the isoelectric point. SDS-PAGE 12.5% was used for the electrophoresis. After that, the gels were stained with Coomassie R-250 dye and then destained in acetic acid and methanol. The gels were performed in triplicate.

The gels were scanned (Image Scanner III, GE Healthcare) and the images were stored for later analysis in the Image Master 2D Platinum program, version 7.0 (GE Healthcare).

E. Statistical Methods

Statistical analyzes were performed using the Statistical Analysis System, version 9.1.3 (SAS) with the PROC MIXED.

III. RESULTS AND DISCUSSION

A. Genotypic and Allelic Frequencies

A total of 155 animals were used in the study. The genotypic frequencies of samples are presented in Table 1.

The allelic and genotypic polymorphisms associated with UOGAST and CAPN obtained from different genetic groups are shown in Table 1.

Table 1. Genotypic and Allelic Frequencies

Marker	**Allelic Frequencies**		**Genotypic Frequencies % (N)**		
UOGCAST	C 0.61	G 0.39	GG 13.6 (21)	CG 51.6 (80)	CC 34.8 (54)
CAPN	C 0.17	T 0.83	TT 69.0 (107)	CT 28.4 (44)	CC 2.6 (04)

B. Proteomic analysis

The image analysis of the 2DE gels (n=32) allowed the identification of 179 spots in common between all gels. Of these, 41 spots showed significant difference between the Volumes Normalized Expression (Figure 1 and Table 1).The analysis of variance of the intensities of expression normalized volumes (VEM) of the spots revealed that: (i) 10 spots showed significant main effect only for the CAPN marker $P < 0.05$ or $P < 0.01$); (ii) 14 spots had significant main effect for the UOGCAST marker ($P < 0.05$ or $P < 0.01$) and (iii) 19 spots had CAPN x UOGCAST marker interaction ($P < 0.05$ or $P < 0.01$).

Lametsch et al. [4] identified 345 spots, of which 103 spots indicate significant changes in expression. Jia et al. [5], working with cattle,

detected 105 spots in total, with 47 spots showed significant expression changes. Bjarnadóttir et al. [6] evaluated the volume changes of protein expression in beef cattle, detected 300 spots and only 35 spots showed significant changes.

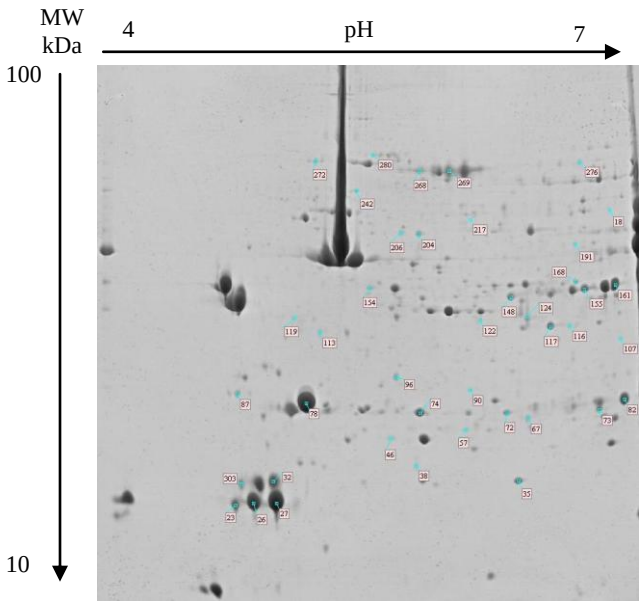

Figure 1. 2DE gel of beef *Longissimus Dorsi* muscle proteins collected 24 hours *post mortem*.

Table 2. Isoeletric point (pI) and Molecular Weight (Mw) of spots identified in Figure 1 (n=41) and that have significant changes in intensity of expression between genotypes.

Spot	pI	Mw (kDa)	Spot	pI	Mw (kDa)
18	**6.93**	**49**	**117**	**6.58**	**29**
23	4.72	15	119	5.07	31
26	4.82	15	122	6.16	30
27	4.96	15	124	6.44	31
32	4.94	16	148	6.35	34
35	6.39	16	154	5.52	35
38	5.79	17	155	6.78	35
46	5.64	19	161	6.96	35
57	6.08	19	168	6.73	36
67	6.45	20	191	6.73	39
72	6.32	21	204	5.80	41
73	6.87	21	206	5.70	41
74	5.81	21	217	6.01	49
78	5.14	21	242	5.43	56
82	7.02	22	268	5.81	62
87	4.73	22	269	5.98	62
90	6.11	22	272	5.19	65
96	5.67	24	276	6.75	65
107	7.00	28	280	5.53	67
113	5.22	29	303	4.75	16
116	6.70	30			

IV. CONCLUSION

1. There were significant changes in intensity of expression normalized volume for the CAPN and UOGCAST markers. This fact suggests the rates of protein expression differed between animals with different genotypes for these markers.
2. Additional studies are needed to verify the mechanism of action of evaluated proteases (calpain and calpastatin) in proteins involved in the tenderness process of Nellore beef cattle.

ACKNOWLEDGEMENTS

To São Paulo Research Foundation (FAPESP) and National Counsel of Technological and Scientific Development (CNPQ) - Brazil

REFERENCES

[1] Koohmaraie, M., Doumit, M.E., & Wheeler, T.L. (1996). Meat toughening does not occur when rigor shortening is prevented. Journal of Animal Science., 74:2935-2942.

[2] Cheong, H.S., Yoon, D.H.; Park, B.L., Kim, L.H., Bae, J.S., Namgoong, S., Lee, H.W., Han, C.S., Kim, J.O., Cheong, I.C. & Shin, H.D. (2008). A single nucleotide polymorphism in *CAPN1* associated with marbling score in Korean cattle. BMC Genetics, 9:33.

[3] Lametsch, R.; Roespstorff, P.; Bendixen, E. (2002). Identification of Protein Degradation during post-morten storage of pig meat. J. Agric. Food Chem., 50:5508-5512.

[4] Lametsch, R. Karlsson, A.; Rosenvold, K.; Andersen, H.J.; Roepstorff, P. Bendixen, E. (2003) Postmortem proteome changes of porcine muscle related to tenderness. J. Agric. Food. Chem. 51:.6992-6997.

[5] Jia, X.; Ekman, M.; Grove, H.; Faergestad, E.M.; Aass, L.; Hildrum, K.I.; Hollung, K.(2007). Proteome changes in bovine Longissimus thoracis muscle during the early postmortem storage period. Journal of Proteome Research, 6:2720-2731.

[6] Bjarnadottir Sg, Hollung K, Faergestad Em, Veiseth-Kent E. (2010). Proteome changes in bovine longissimus thoracis muscle during the first 48 h postmortem: shifts in energy status and myofibrillar stability. J Agric Food Chem. 58:7408–14.