Proteome changes of beef in Nellore cattle (*Bos indicus*) with different genotypes for tenderness

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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 (CAP) and calpastatin (CAST) genes. The 2DE was carried out in LM samples collected 24 h post mortem for each genotype group. The genotypic frequencies for CAP were TT=69.06%; CT=28.4% and CC=2.6% and CAST 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) and were identified by mass spectrometry; 11 were metabolic enzymes, 5 stress and defense proteins, 15 structural proteins and 10 were classified like "others". In conclusion, there were differences in protein profile of different genotypes, but more studies are necessary to elucidate how these differences can affect Nellore cattle 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 two-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 proteomic analysis.

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 raised on pasture and 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. Between the 12th and 13th ribs, three steaks were removed from the *Longissimus* muscle at 24 hours post mortem , two inches thick each. They were vacuum packaged and aged at 2°C for 1, 7 or 14 days. After each period, the Warner-Bratzler Shear Force (WBSF) determination was made (not frozen samples). Simultaneously, one gram of sample was collected for proteomic analysis.

B. Marker Used and Genotyping

Cattle were genotyped for a single nucleotide polymorphism (SNP) in the calpain (CAP 4751; GeneBank accession number: AF248054, position 6545) and calpastatin genes (CAST; GeneBank accession number: AY008267, position 282).

Blood samples were collected and DNA isolated from these animals was storage -80ºC. The CAP and CAST 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. Meat Tenderness

The meat tenderness was determined by Warne Bratzler Shear Force (WBSF), according American Meat Science (1995) procedures.

E. Proteomic analysis

The 2DE was carried out in *Longissimus Dorsi* muscle samples (1g) collected 24 h *post mortem* for each genotype. The protein extraction was performed according Carvalho *et al* [4]. 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 analyzed in the Image Master 2D Platinum program, version 7.0 (GE Healthcare). The spots that were detected with differential expression were excised from the gels and digested with trypsin solution for MALDI-TOF/TOF MS/MS analysis. The spectra were processed using ABSciex MALDI TOF/TOF series Explorer version program and the dates were analyzed with Protein Pilot with MASCOT. For the protein identification, was used UniProtKB/Swiss-Prot database for *Bos taurus*.

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, Allelic Frequencies and Meat Tenderness

A total of 155 animals were used in the study. The allelic and genotypic polymorphisms associated with CAST and CAP obtained from different genetic groups are shown in Table 1.

Both alleles of CAST polymorphism were observed in all animals evaluated and had different frequencies (C = 0.61 and G = 0.39), however in the CAP polymorphism the Allele C frequency (0.17) was less than T allele (0.83). The genotypic frequencies of CAST marker were well distributes among genotypes and for CAP the genotypic distributions were asymmetric with low frequency of favorable genotype (CC).

The meat tenderness of *Longissimus* muscle was evaluated by each genetic group and aging period (until 14 days). It's was observed overall increased of tenderness during the aging period independent of genetic group. In addition, it was detected effect ($P<0.05$) of CAST marker with 14 days of aging, where the favorable genotype (CC) had minor values of WBSF (6.4 kg) while the

unfavorable genotype had more 1.3 kg of WBSF values (Table 2). The results confirm the efficient of this marker for *Bos indicus* cattle.

Table 1. Genotypic and Allelic Frequencies

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

Witch respect of marker, despite of low frequency of the favorable allele CC, it was observed effect (P<0.05) for aging meat until 7 days. The genotype CT was considered intermediary for tenderness (Table 2).

Table 2. Mean and standard error of Warner Bratzler Shear Force (WBSF) values by molecular maker and aging time.

Marker	Genotype	*Postmortem aging, d*		
		1 day*	7 days*	14 days*
CAST	CC	9.7 ± 0.3^{A}	8.1 ± 0.2^{A}	6.4 ± 0.2^{B}
	CG	9.3 ± 0.2^{A}	8.0 ± 0.2^{A}	6.8 ± 0.2^{B}
	GG	9.1 ± 0.4^{A}	8.5 ± 0.4^{A}	7.7 ± 0.4^{A}
CAP	CC	7.5 ± 0.9^{B}	6.2 ± 0.9^{B}	6.3 ± 0.8^{A}
	CT	9.5 ± 0.3^{A}	7.9 ± 0.3^{AB}	6.5 ± 0.2^{A}
	TT	9.5 ± 0.2^{AB}	8.2 ± 0.2^{A}	6.9 ± 0.1^{A}

*N=155; Different superscripts within column and genotype category indicate differences (P<0.05)

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).The analysis of variance of the intensities of expression normalized volumes (VEM) of the spots revealed that: (i) 8 spots had significant main effect for CAP marker (P <0.05 or P <0.01); (ii) 11 spots had significant main effect for CAST marker (P <0.05 or P <0.01) and (iii) 20 spots had CAP x CAST marker interaction (P <0.05 or P <0.01).

Lametsch *et al* [5] identified 345 spots, of which 103 spots indicate significant changes in expression. Jia *et al* [6], working with cattle, detected 105 spots in total, with 47 spots showed significant expression changes. Bjarnadóttir *et al* [7] evaluated the volume changes of protein expression in beef cattle, detected 300 spots and only 35 spots showed significant changes.

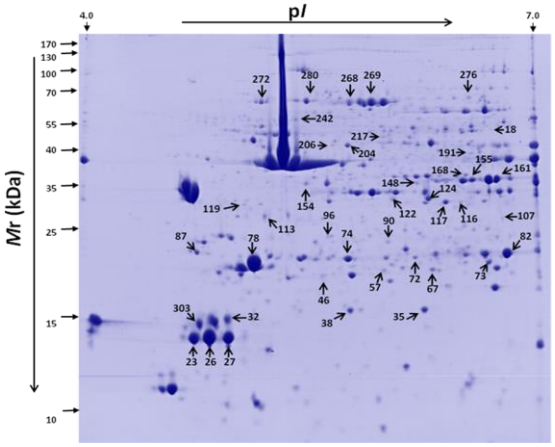

Figure 1. 2DE gel of beef *Longissimus Dorsi* muscle proteins collected 24 hours *post mortem*.

The mass spectrometry analysis showed the presence of 11 metabolic enzymes, 5 stress and defense proteins, 15 structural proteins and 10 classified like "others". Inside the metabolic enzymes group were identified 2 fragments of Triosephosphate isomerase (TPI1), 2 of Malate dehydrogenase (MDH1), 3 of beta-enolase (ENO3), 2 of creatine-kinase (CKM), 1 of Guanidinoacetate N-methyltransferase (GAMT) and 1 to phospoglycolate phosphatase (PGP).

Witch respect of stress and defense proteins were found 1 fragment of Heat Shock Protein beta-6 (HSPB6), 3 of Heat Shock Protein 27 (HSP27) and 1 of Heat Shock Protein 70 (HSP70).

Also, were found a large number of structural proteins like one sub-units of F-actin (CAPz), 7 of Myosin light chain (MYL6B and MYL1), 2 of Actin (ACTA1), 5 of Troponin T (TNNT1) and 1 of KBTBD10 Protein.

Table 2. Results of Mass spectrometry analysis with Theoretical (To) and Experimental (Ex) Isoeletric point (pI) and Molecular Weight (Mw) of spots identified in Figure and that have significant changes in intensity of expression between genotypes.

Spot	Abrev Gene Name	To pI/Mr	Ex pI/Mr	Marker Effect
18	ENO3	7.6/47.1	6.6/39.1	Cap X Cast
23	MYLPF	4.9/19.0	4.6/14.6	Cap X Cast
26	MYLPF	4.9/19.0	4.8/14.6	Cap X Cast
27	MYLPF	4.8/19.0	4.9/14.6	Cap X Cast
32	MYL2	4.8/18.9	4.9/15.6	Cap X Cast
35	HSPB6	5.9/17.5	6.3/15.7	Cap
38	-	-	5.7/16.9	Cap
46	MYL6B	5.4/23.4	5.6/18.9	Cast
57	HSPB1	6.0/22.4	6.0/19.7	Cast
67	TPI1	6.4/26.7	6.3/20.5	Cap X Cast
72	HSP27	6.0/22.4	6.2/20.9	Cap X Cast
73	CKM	6.6/43.0	6.7/21.2	Cap X Cast
74	HSP27	6.0/22.4	5.74/21.0	Cap X Cast
78	MYL1	4.7/19.5	5.1/20.4	Cast
82	TPI1	6.4/26.0	6.9/21.6	Cap
87	YWHAE	4.6/29.2	4.7/21.8	Cap X Cast
90	GAMT	5.7/26.6	6.0/22.8	Cast
96	ACTA1	5.2/42.0	5.6/24.0	Cast
107	CKM	6.6/43.0	6.9/27.7	Cap X Cast
113	(CAPz b)	6.0/33.7	5.1/28.3	Cap
116	TNNT1	5.7/31.3	6.5/30.3	Cast
117	TNNT1	5.7/31.3	6.4/30.2	Cast
119	PGP	5.2/34.3	5.0/30.8	Cap, Cast
122	MDH1	6.2/36.4	6.1/31.9	Cap
124	MDH1	6.2/36.4	6.3/31.6	Cast
148	ENO3	7.6/47.1	6.2/34.4	Cap X Cast
154	ACTA1	5.2/42.0	5.5/35.0	Cap X Cast
155	TNNT3	6.0/32.1	6.6/35.3	Cap X Cast
161	TNNT3	6.0/32.1	6.8/35.4	Cap X Cast
168	-	9.2/58.1	6.6/36.0	Cap
191	ENO3	7.6/47.1	6.58/39.1	Cast
204	UQCRC1	5.9/52.7	5.7/41.6	Cap X Cast
206	UQCRC1	5.9/52.7	5.6/41.5	Cap, Cast
217	-	6.6/57.7	6.0/45.6	Cap X Cast
242	-	-	5.4/55.3	Cast
268	HSP70-2	5.6/70.2	5.7/62.4	Cap
269	ALB	5.9/69.3	5.9/62.5	Cap X Cast
272	KBTD10	5.1/68.1	5.1/63.8	Cast
276	TF	7.1/77.7	6.6/64.7	Cap
280	-	-	5.5/66.9	Cap X Cast
303	MYL2	4.8/18.9	4.7/15.4	Cap X Cast

IV. CONCLUSION

There were significant changes in intensity of expression normalized volume for the CAP and CAST markers. This fact suggests the rates of protein expression differed between animals with different genotypes for these markers. Additional studies are needed to verify the mechanism of action of evaluated proteases in the tenderness process of Nellore beef cattle.

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