CALPASTATIN ACTIVITY AND BEEF TENDERNESS OF NELLORE AND ANGUS CATTLE FED TWO FEEDING STRATEGIES

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Abstract – Calpastatin activity was measured on longissimus muscle of 17 Nellore and 17 Angus cattle fed two different diets. Cattle were randomly assigned into one of the following feeding strategies: 100:0 concentrate:roughage ratio (9 Nellore and 9 Angus) and 70:30 concentrate:roughage ratio (8 Nellore and 8 Angus) fed *ad libitum*. *Longissimus* muscle (LM) samples were collected 24 h postmortem and calpastatin activity measured. In order to validate calpastatin results, Warner-Bratzler shear force (WBSF) and myofibrillar fragmentation index (MFI) were performed on longissimus muscle samples also collected 24 h postmortem from each animal. Higher calpastatin activity was observed on LM from Nellore cattle (*P* = 0.0238). This result was confirmed by the lower values of MFI on LM of Nellore cattle (*P* = 0.0001) which led to a greater WBSF value (*P* = 0.0026) of Nellore compared to Angus Beef. Calpastatin activity was not affected by feeding management (*P* = 0.8437). Similarly, no differences were observed among feeding regimes for MFI (*P* = 0.8300) and WBSF (*P* = 0.9079). These results indicate that calpastatin is a main factor responsible for toughness of Nellore beef. Moreover, the use of diets containing high grain rations does not increase proteolysis postmortem.

Keywords - Bovine, Breed, Beef, Calpain, Diet, Meat

I. INTRODUCTION

The calpain-calpastatin system is the major proteolytic system that affects muscle fiber degradation during postmortem aging and consequently affects beef tenderness (Taylor *et al* 1995). In this context, the lack of tenderness of Brazilian beef is related to the fact that the breed mainly originated from *Bos indicus* cattle, which is recognized to produce less tender meat due to a lower calpain activity compared to *Bos taurus* cattle (Koohmaraie, 1994). However, most of the studies comparing calpain-calpastatin activity and its relationship with tenderness of beef from *Bos indicus* and *Bos taurus* have used breeds other than Nellore cattle that are usually raised in non-tropical conditions as a model. Thus, since Nellore is the main beef breed used in Brazil, the conclusions reported so far may not represent the causes underlying the low tenderness of Brazilian beef. Moreover, different feeding strategies are used in beef production in Brazil due to the seasonal variation of quantity and quality of forage which represents the lowest cost feed source in tropical areas. Consequently, differences in meat tenderness may also occur due to effects of feeding level which also affects proteolysis postmortem by changing the calpain-calpastatin system activity. As such, we aimed to investigate differences in calpastatin activity and beef tenderness of Nellore compared to Angus cattle fed different feeding levels to outline a more representative data of factors underlying Brazilian beef tenderness.

II. MATERIAL AND METHODS

Thirty-four animals with average body weight of 350 kg and 20 months of age were used. Cattle were confined in individual pens for a total experimental period of 84 days. Animals were assigned into a completely randomized 2 x 2 factorial design with the factors being two

breeds (Nellore and Angus) and 2 dietary treatments. Dietary treatments consisted of 100:0 concentrate:roughage (9 Nellore and 9 Angus), and 70:30 concentrate:roughage (8 Nellore and 8 Angus) fed *ad libitum*. The feeding strategies used were chosen to be as representative as possible of the feeding conditions commonly observed in Brazilian beef systems. Chemical composition and ingredient proportion of the experimental diets are presented in Table 1.

Table1 Ingredient proportion and chemical composition of the experimental diets

Item	Concentrate : roughage ratio	
	100 : 0	70 : 30
Ingredient proportion, % of dry matter		
Corn grain	85.0	-
Protein-mineral premix[1]	15.0	-
Corn silage	-	30.0
Corn meal	-	58.0
Soybean meal	-	10.0
Mineral mixture	-	2.0
Chemical composition, % of dry matter		
Dry matter	88.0	72.0
Crude protein	12.5	12.4
Neutral detergent Fiber	11.9	26.2
Total digestible nutrients	81.0	78.1
Starch	62.5	49.7

[1] Crude protein = 32.0%; Total digestible nutrients = 50.0%; Ca = 45.0 g/kg; Mg = 7.5 g/kg; P = 11.0 g/kg; Cu = 104 mg/kg; Zn = 344 mg/kg; Se = 0.83 mg/kg; Virginiamycin = 140.0 mg/kg; Monensin = 120.0 mg/kg

At the end of the experimental period (84 d) all the animals were slaughtered. Ante mortem handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997). *Longissimus* muscle samples were collected after 24 h postmortem chill (2ºC). Calpastatin activity was measured according to the method described by Koohmaraie (1990). Myofibrillar fragmentation indices (MFI) were determined on fresh muscle according to Culler et al. (1978). Warner-Bratzler shear force (WBSF) was performed following the AMSA (1995) guidelines.

The statistical analysis was performed using the PROC GLM of SAS 9.1 (2003) in a 2 x 2 factorial design, with two breeds and two feeding levels, using type III sum of squares and assuming a significance level of 5%.

III. RESULTS AND DISCUSSION

No interactions between breed and feeding levels were observed ($P > 0.05$) for any of the variables evaluated. Thus, effects of breed and feeding strategy are discussed independently.

Proteolytic enzyme activity participates in myofibrillar breakdown during normal protein turnover and continues to be active in muscle postmortem influencing tenderization of meat. Among other proteolytic enzymes, calpains causes a hydrolysis of intermediate filaments (titin and nebulin) weakening the muscle fibers structure and has its activity inhibited by calpastatin (Goll et al., 2003).

In the present study calpastatin activity (CA) was higher in LM muscle from Nellore than Angus cattle ($P = 0.0238$; Figure 1).

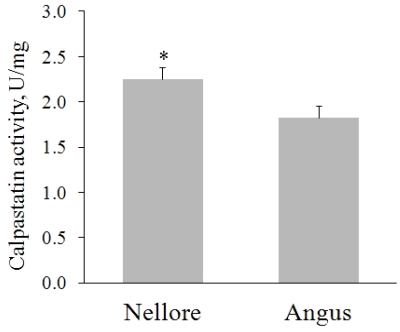

Figure 1. Calpastatin activity measured 24 h postmortem on *longissimus* muscle of Nellore and Angus cattle.

Thus, the greater activity of calpastatin likely led to a decreased in calpain activity in LD muscle from Nellore compared to Angus cattle since a lower postmortem myofibrillar fragmentation index (MFI) was observed in LD muscle from Nellore cattle (P = 0.0001; Figure 2).

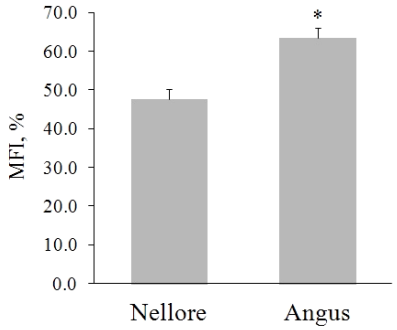

Figure 2. Myofibrillar fragmentation index (MFI) measured 24 h postmortem on *longissimus* muscle of Nellore and Angus cattle.

Consequently, greater values of WBSF (P = 0.0026; Figure 3) was observed in beef from Nellore cattle.

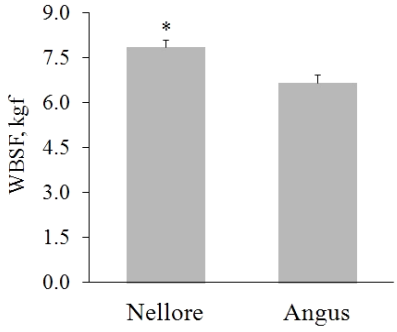

Figure 3. Warner-Bratzler shear force (WBSF) of beef from Nellore and Angus cattle 24h postmortem.

These results suggest that the greater calpastatin activity is the main factor responsible for the lower tenderness of Nellore cattle. Moreover, similarly to what has been previously reported (Koohmaraie et al., 1994), these results confirm that a lower tenderness is observed in *Bos indicus* cattle regardless of the breed type.

Besides genetics, calpastatin activity is also regulated by environmental factors such as diet (Du et al., 2004). However, no differences were observed (P = 0.8437) for calpastatin activity measured on LD muscle of cattle fed different concentrate:roughage (Figure 4).

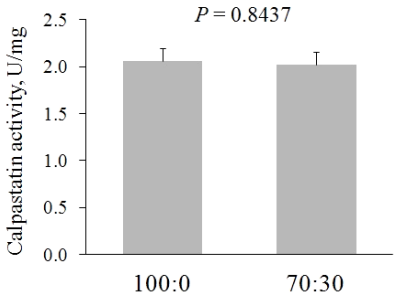

Figure 4. Calpastatin activity measured 24h postmortem on longissimus muscle of cattle fed 100:0 and 70:30 concentrate:roughage diets.

As no differences in calpastatin activity was observed, similar values of MFI were found (P = 0.8300) on the *longissimus* muscle of cattle fed both concentrate:roughage ratios (Figure 5).

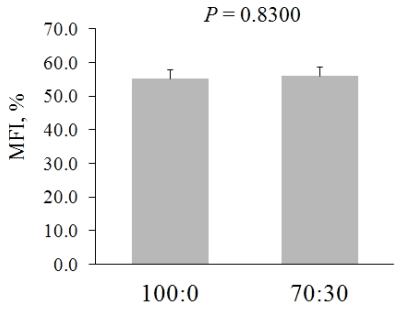

Figure 5. Myofibrillar fragmentation index (MFI) measured 24h postmortem on longissimus muscle of cattle fed 100:0 and 70:30 concentrate:roughage ratio.

The regulation of calpastatin activity through the nutritional management of the animal regulates protein accretion in skeletal muscle. It has been demonstrated that feed restriction of cows leads to a greater calpastatin activity (Du et al, 2004).

Such behavior occurs due to increased myofibrillar protein turnover, as well as to decreased protein synthesis following nutrient restriction. This scenario also may occur as a result of the mobilization of aminoacids to be used as a substrate for energy production by the organism. However, even though different concentrate proportions were used in this trial, both feeding managements has not characterized a feed restriction that would result in a decrease of calpastatin activity. Because no differences were observed for calpastatin activity, similar values of WBSF (P = 0.9079) were observed for cattle fed at both feeding managements (Figure 6).

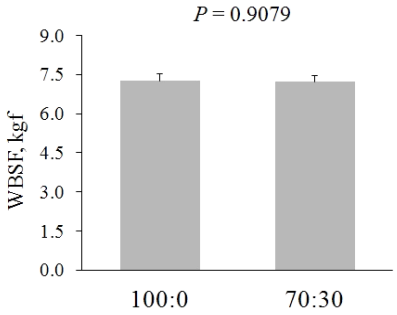

Figure 6. Warner-Bratzler shear force (WBSF) measured 24h postmortem on longissimus muscle of cattle fed 100:0 and 70:30 concentrate:roughage.

IV. CONCLUSIONS

These data shows a substantially contribution of calpastatin activity on toughness of beef from Nellore compared to Angus cattle under tropical conditions. Moreover, the use of 100:0 or 70:30 concentrate:roughage does not affect proteolysis postmortem leading to similar tenderness of beef.

ACKNOWLEDGMENTS

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