ELISA Test for Calpastatin

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The inconsistency in meat tenderness is one of the major problems facing the meat industry (Morgan et al., 1991; Morgan, 1992; Savell and Shackelford, 1992). The National Beef Quality Audit-1995 identified tenderness among the top 10 concerns of retailers, restaurateurs and purveyors. This is due to a great variation in the extent of post-mortem tenderization in beef and is intensified by the inability to accurately classify carcasses based on ultimate meat tenderness. Thus, there is a pressing need to develop strategies to predict beef tenderness and quality.

It is now accepted that calpastatin, the endogenous and specific inhibitor of calpain enzymes, inhibits calpain activity in post-mortem meat and thereby regulates the rate and extent of post-mortem tenderization (Koohmaraie et al., 1995). It has been repeatedly observed that that calpastatin activity at 1 d post-mortem can account for up to 30% to 40% of the variation in beef tenderness. This implies that calpastatin activity could be measured in samples from carcasses at 24 to 48 hours post-mortem and utilized to predict tenderness. However, current procedures for quantification of skeletal muscle calpastatin are time-consuming and laborious. This limitation hinders the implementation of such an application at the packer level.

Koohmaraie described the development of an Enzyme-Linked Immunosorbent Assay (ELISA) procedure to rapidly and accurately quantify skeletal muscle calpastatin activity in meat. This approach to quantifying calpastatin removes some of the limitations for implementation of a calpastatin-based method for predicting tenderness.

The objectives of this session were to describe the following steps in the development and application of an ELISA for calpastatin: 1) Express and purify recombinant bovine skeletal muscle calpastatin. 2) Raise polyclonal antibodies against recombinant calpastatin. 3) Develop an ELISA protocol to quantify skeletal muscle calpastatin. 4) Determine the efficacy of the calpastatin ELISA in prediction of meat tenderness. An additional objective was to introduce an alternate approach, utilizing a shear force measurement at 2 days post-mortem, to accurately classify beef based on ultimate tenderness.

Description of an ELISA

The method described is an indirect antibody capture procedure. Briefly, the antigen is attached to a solid phase support (such as a microtiter plate well) and the primary antibody which is specific for the antigen (in this case, skeletal muscle calpastatin) binds to the antigen. Primary antibody bound to the antigen is quantified by a secondary antibody (specific for the primary antibody) which is conjugated to horseradish peroxidase. Quantification of the amount of secondary antibody bound to the primary antibody is achieved by exposing a horseradish peroxidase substrate and spectrophotometrically determining the amount of product produced by the horseradish peroxidase enzyme.

Production of Antigen and Characterization of Polyclonal Antibodies

A recombinant product corresponding to domains 2, 3 and 4 of bovine skeletal muscle calpastatin was expressed in culture and purified. The purified product was utilized as an antigen to raise polyclonal rabbit anti-recombinant calpastatin antibodies at the Monoclonal/Polyclonal Antibody Core Facility, Center for Biotechnology (University of Nebraska, Lincoln). Western blot analysis of pre-rigor skeletal muscle extracts revealed that these monospecific polyclonal antibodies recognize an immunoreactive calpastatin band that migrates at 130 kDa on SDS-PAGE . Specific details of sample preparation and ELISA protocol are described by Doumit et al. (1996).

Quantification of Calpastatin

Calpastatin ELISA results were linearly related to calpastatin activity of heated longissimus muscle homogenates from pre-rigor lamb skeletal muscle (r^2 = .89) and post-

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rigor beef samples aged for 48 h (r^2 = .90). The assay was very repeatable (r^2=.97). The intra-assay CV was < 5% and inter-assay CV was < 6% . Therefore, the assay was verified as a suitable analysis to quantify calpastatin .

Efficacy of Calpastatin ELISA in Prediction of Warner-Bratzler Shear Force

Calpastatin quantification by ELISA is more rapid and more sensitive than conventional activity assays. This affords the possibility of determining the relationship of calpastatin to meat tenderness on greater number of carcasses. The relationships of calpastatin ELISA quantification at 2 d post-mortem to Warner-Bratzler shear force at 14 d post-mortem in lamb (r^2=.44) and beef (r^2=.28) were reported to be similar to the relationship of calpastatin activity to shear force (Doumit and Koohmaraie, 1996). The reason for the better relationship of calpastatin quantification by ELISA in lamb was suggested to be the greater range in shear force in the sample population of lambs when compared to the beef in this study.

Koohmaraie stated that variation in calpastatin, by itself, does not account for enough variation in beef tenderness to be used as an effective predictor of meat tenderness. Furthermore, calpastatin ELISA quantification, though less time-consuming than the conventional assay, is not sufficiently rapid to be implemented as a measure to predict meat tenderness at the commercial level.

Tenderness-Based Classification of Beef

Given the conclusion that no singular characteristic can predict meat tenderness, researchers at the Meat Animal Research Center have developed the concept that Warner-Bratzler shear force at 2 d post-mortem can be used as a criterion to predict tenderness of aged beef. This approach was reported to classify beef carcasses – within about 90% accuracy – into assigned categories of guaranteed tender, probably tender and probably tough.

Koohmaraie reported that this relationship has prompted efforts to engineer a system to rapidly measure shear force. Briefly, the prototype procedure would be as follows:

1. Carcasses are chilled 48 hours post-mortem.
2. A 2.54 centimeter thick steak is removed between the 12th and 13 rib.
3. The steak is placed on a belt conveyor and advances to a water-jet trimming system to separate the fat and bone.
4. The steak is placed on a continuous feed cooker and is cooked to 71°C in 6 minutes.
5. A slice which is parallel to the orientation of the muscle fibers is removed from the cooked steak.
6. The slice is then sheared, using a modified shear force device.

It was estimated that this process can be completed in 10 minutes in an on-line system. A crucial point of this procedure is that the steaks must be a consistent thickness or the endpoint temperature among steaks will not be uniform.

It was reported that the system was at least as repeatable as conventional shear force evaluations. Moreover, it was suggested that the consistent cooking endpoint results in a more accurate, more repeatable assay. Koohmaraie acknowledged that costs would be a factor in the implementation of this system, but predicted that accurate classification of beef based on tenderness would add value to the carcasses.

Summary

A rapid, sensitive calpastatin ELISA assay was developed and determined to quantify calpastatin activity accurately. This methodology will complement studies designed to evaluated the role of calpastatin in protein turnover in muscle and in the post-mortem aging process. This calpastatin assay explains the same amount of variation of beef tenderness as the conventional calpastatin activity assay. It was therefore concluded that calpastatin ELISA quantification, by itself, will not be effective in accurately classifying carcasses based on meat tenderness. Direct measurement of shear force offers an alternate approach to categorizing beef carcasses based on tenderness. Although invasive, a tenderness-based approach to categorize beef appears to be a promising avenue toward guaranteed tenderness.

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