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REGRESSION ANALYSIS OF DESMIN AND TROPONIN-T DEGRADATION AND FEEDING REGIMES TO TENDERNESS OF THE LONGISSIMUS OF CULL COWS

L. W. Kutzler*, A.C. Kelly, S. F. Holmer, F.K. McKeith, and J. Killefer

University of Illinois at Urbana-Champaign

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Introduction

Last year 2.7 million cull beef cows and 2.3 million cull dairy cows (USDA-NASS, 2005) were harvested, which accounts for 16.7% of the total cattle slaughtered in the United States. This aged cow population represents an area of the meat industry that is under utilized. Commonly, meats from these animals are used in lower quality products such as ground beef. As the cow ages, physiological changes occur that decrease the tenderness of the meat. Including, changes in the collagen content (Bosselmann et al., 1995) and possibly the endogenous protease population as well. Postmortem **(PM)** proteolysis of muscle proteins also plays an important role in meat tenderness. Degradation of Desmin, located on the periphery of the myofibrillar Z-disk (Price and Schweigert, 1987), is involved with PM tenderization (Hwan and Bandman, 1989; Whipple and Koohmaraie, 1991), as well as Troponin-T degradation, which is associated with the thin filament (Price and Schweigert, 1987). The extent of degradation of these structural and peripheral proteins could affect the progression of PM tenderization (Huff-Lonergan, 1996). In purchasing meat consumers place tenderness as one of the most important factors (Miller et al., 1995), affecting acceptability and how much they are willing to pay for the product (Lusk et al., 2001). To help improve the value of cull cow carcasses, cows are finished on high energy diets. This practice increases muscling and steak tenderness (Schnell et al., 1997). Another potential method to increase muscling is with the addition of Optaflexx™ (ractopamine HCl).

Objectives

Determine the correlation between percent shear force decrease and Troponin-T and Desmin degradation in non-dairy type cull cows finished on forage maintenance diet, high energy concentrate diet, and high energy plus Optaflexx™.

Methodology

Live Phase

Animals were raised per approved University of Illinois protocols. Cows were allotted to pens (twelve pens of five animals) based on body weight, hip height, body condition score and ultrasonic backfat thickness. Dietary treatments consisted of a forage maintenance diet (CON), high energy concentrate diet (FED), and high energy plus Optaflexx™ (OPTA). Cows were harvested in four groups (1 pen per treatment, 15 animals total) over four consecutive weeks. Therefore, after the first harvest group started on trial, each following week another group of three pens was placed on trial. All cows were fed for 57d, with the OPTA group receiving Optaflexx™ at 200 mg/head/d for the last 35d (fed according to label directions). FED and OPTA groups were adapted to the final diet over a 3 week period.

Harvest

After 57d on trial, each harvest group of cows was transported to the University of Illinois Meat Science Abattoir the night before harvest. During lairage, cows had full access to water, but were held without feed. The following morning, all cows in the group were harvested in random order.

Each cow was weighed to obtain a live weight, immobilized with a captive bolt stunner, exsanguinated, and head removed. The hide was removed and the carcass was eviscerated. After evisceration, carcasses were split, washed, weighed, and placed into a 4ºC cooler. Observations were made and recorded regarding any cows that were pregnant or any carcasses that were condemned due to sickness.

Ageing Samples

At 2d post-mortem, the ribeye from both sides of the carcass had a 2.5cm section removed to expose a fresh surface. Two 2.5cm steaks were cut from each ribeye. These steaks were randomly assigned to ageing periods of 2, 7, 14 and 21d post-mortem. Steaks within the same ageing period were vacuum packaged together, boxed and stored at 4ºC until the appropriate ageing time, at which point they were frozen at -30ºC until shear force analysis could be completed.

Shear Force Determination

Steaks used for Warner-Bratzler shear force determination were thawed overnight at 4ºC, trimmed to a uniform size and cooked on a Farberware open-hearth grill (Model 455N, Walter Kidde, Bronx, NY). Internal temperature was monitored using copper-constantan thermocouples (Type T, Omega Engineering, Stanford, CT) connected to a digital scanning thermometer (Model 92000-00 Barnant Co., Barington, IL). Steaks were cooked on one side to an internal temperature of 35ºC, turned over and cooked to a final internal temperature of 70ºC. After cooking, steaks were allowed to cool to 25°C before four 1.3cm cores were removed parallel to the orientation of the muscle fibers. Cores were sheared on an Instron® universal testing machine (Model 112) set with a 10kg load

cell and a 200mm per minute chart drive and crosshead speed. Shear force was determined for each core, and these values were averaged for each sample.

Tissue Preparation

Proteomic analysis was performed on 51 *Longissimus* muscle samples. Day 0 samples were obtained by biopsy from the 9-10th rib region at 1.5h post-mortem, snap frozen in liquid nitrogen and stored at -80^{o}C. Aged samples were collected from steaks aged for 13d post mortem and then frozen at -80^{o}C until analysis. Myofibrillar proteins were extracted as described by Kent and coworkers (2004) with some modifications. In brief, frozen muscle portions were powdered in liquid nitrogen and a 200mg sample was transferred to a 1.5ml microcentrifuge tube. Five volumes (v:w) of extraction buffer (100mM Tris-HCL [pH 8.3] and 5 mM EDTA) were added to the tube and homogenized with an Ultra-Turrax (IKA Werke, Germany) set to high for 15sec, vortexed, then homogenized again for 15sec. This was all completed on ice. Two-hundred microliters of the fraction were prepared for SDS-PAGE with the addition of an equal volume of treatment buffer (.125 M Tris-HCl [pH 8.6], 4% SDS, and 20% glycerol). The suspension was then heated at 70^{o}C for 20min and centrifuged at 15,000 x g for 30min. Supernatant was collected and stored at -80^{o}C for further analysis. Protein concentrations were determined with the Pierce BCA (Pierce Biotechnologies, USA) microplate protein assay according to manufacturer's protocol. Fractions were diluted 15-fold with distilled water so that the concentration would fall within a readable range. Bovine serum albumin was used to generate a standard with 7 points between 2mg/ml and 0.1mg/ml. Standards were made fresh daily from a 2mg/ml stock and each assayed plate contained its own standard curve. Protein concentrations from the fractions were adjusted to 5mg/ml for SDS-PAGE.

SDS PAGE

Protein electrophoresis was carried out using precast NuPAGE 10% Bis-Tris gels with MOPS running buffer (Invitrogen, USA). Loaded samples contained 20ug protein per lane and were prepared per NuPAGE protocol (Invitrogen, USA). Magicmark XP (Invitrogen, USA) was loaded for a chemiluminescent molecular weight standard. Gels were run on a water-cooled Hoefer SE260 (Hoefer, USA) at a constant voltage of 180v for 90min.

Western Blotting

After electrophoresis gels were transferred onto PVDF 0.45um membranes (Invitrogen, USA) in a Trans-Blot Cell (Bio-Rad, USA) for 3h at a constant voltage of 30v. Membranes were processed for chemiluminescent detection using the Western Breeze Immunodetection kit (Invitrogen, USA). Membranes were blocked for 30min and then probed with Desmin antibody D1033 at 1:500 (Sigma, USA) and Troponin-T antibody T6277 at 1:1000 (Sigma, USA) for 60min. Membranes were washed for 45min, probed with alkaline phosphatase goat anti-mouse secondary antibody for 30min and washed again for 45min. Membranes were incubated with substrate for 5min and visualized using the ChemiGenius^{2} Imaging System (Syngene, UK). Images were analyzed using GeneTools (Syngene, UK). Changes in Desmin and Troponin-T protein

profiles were determined by the percent decrease in intact protein from 0 to 13d per animal and correlated to percent decrease in shear value from 2 to 13d.

Statistical Analysis

Data were analyzed with the MIXED procedure in SAS (1999) using the lsmeans statement for means and standard error calculation. The pdiff option was used for determining significance. When determining significance for the effect of dietary treatment, only the comparisons of Control vs. Fed and Fed vs. Fed + Optaflexx™ were evaluated. Significance was determined at $P > 0.10$ = NS, $P \leq 0.10$ = †, $P \leq 0.05$ = *, $P \leq 0.01$ = **, $P \leq 0.001$ = ***. For all data, Pen (5 cows/pen) served as the experimental unit. Regression analysis was performed with REG procedure in SAS (1999).

Results & Discussion

Initial shear force values were lower with the FED and OPTA compared to CON ($P<0.05$), however, there were not any differences in the final shear force values ($P>0.10$; Table 1). This suggests that meat from forage fed cows can be of equal tenderness when aged for a sufficient period of time. Day 7 and 14 shear values were numerically lower in the FED and OPTA treatments indicating that high energy diets can potentially decrease the time needed for ageing.

SDS-PAGE of 20ug of 0 and 13d *Longissimus* total protein preparations yielded similar protein profiles (Fig. 1). Visualization of the Desmin Western blot displayed an undegraded band at 55 kDa at 0d and three proteolytic fragments of 49, 47 and 40 kDa (Fig. 2) and decreased undegraded band intensity (Fig. 3). Enzymatic degradation of Desmin from 0 to 13d was not affected by the three feeding regimes ($P>0.10$; Table 2). Desmin degradation was chosen as an indicator of PM proteolysis because it is a specific substrate for the calpain system (Taylor et al., 1995). Also, other proteolytic systems do not have access to, or capabilities to degrade Desmin (Goll et al., 1992; Koohmaraie, 1992). Analysis of the Troponin-T Western blot yielded an intact doublet band of 37 kDa at 0d, and proteolytic fragments of 35 and 32 kDa (Fig. 4), and decreased intact band intensity on 13d (Fig. 5). When comparing Troponin-T degradation from 0 to 13d in the CON group to the FED group, the CON cows had a higher percent difference ($P=0.07$) (Table 2). Given the high coefficient of determination, Troponin-T may serve as a good indicator of PM proteolysis. Taylor and co-workers (1995) demonstrated that during PM proteolysis myofibrils are broken at their I-bands. Because of this, it is possible that when Troponin-T, a regulatory component in the actin-myosin complex breaks down, the thick and thin filament interactions are altered, which can be a indicator of tenderness (Huff-Lonergan et al., 1996).

The coefficient of determination (R^2) between Desmin and Troponin-T degradation from 0 to 13d and percent shear force decrease from 2 to 14d were 0.68 and 0.59 respectively. Coefficient of determination with combined Desmin and Troponin-T degradation against percent shear force decrease was 0.75 (Table 3).

Conclusions

Overall, Desmin demonstrated a greater coefficient of determination (R^2) than Troponin-T, yet both demonstrated an additive effect when combined and plotted against percent shear force decrease. Initial shear force values were lower for FED and OPTA groups compared to CON, but feeding regimes did not affect the final shear force value, nor the percent Desmin degradation. However, percent Troponin-T degradation was higher in the CON cows compared to the FED cows (P=0.07). FED and OPTA treatments showed the potential to decrease the time needed to age samples.

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Tables and Figures

Table 1. Cull Cow Ageing Samples: [1] Warner-Bratzler shear force

Time	CON (C)	FED (F)	OPTA (O)	C vs. F *P*- value	F vs. O *P*-value	SEM
Pens, n	4	4	4	--	--	--
2-d Ageing	7.11	6.30	5.91	*	NS	0.50
7-d Ageing	5.90	5.73	5.40	NS	NS	0.55
14-d Ageing	5.14	4.72	4.78	NS	NS	0.63
21-d Ageing	4.73	4.36	4.42	NS	NS	0.56

NS > 0.10, † ≤ 0.10, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001

[1] Steaks for ageing were cut from the ribeye from both sides of the carcass and randomly assigned to the appropriate times

Table 2. Cull Cow Ageing Samples: % Protein Degradation From 0d to 13d in the *Longissimus*

Protein	CON (C)	FED (F)	OPTA (O)	C vs. F *P*- value	F vs. O *P*-value	SEM
Desmin	62%	56%	53%	NS	NS	0.07
Troponin-T	49%	33%	43%	†	NS	0.07

NS > 0.10, † ≤ 0.10, * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001

Table 3. Cull Cow Ageing Samples: Regression Analysis in *Longissimus* samples[A]

Protein	Equation[B]	R^2
Desmin	y = .433d + .019	0.68
Troponin-T	y = .3902t + .103	0.59
Desmin & Troponin-T	y = .297d + .180t + .021	0.75

[A] % Protein degradation to % reduction in shear force

[B] y = % decrease in shear force, d = % Desmin degradation, t = % Troponin-T degradation

Fig 1. Representative SDS-PAGE of total protein isolates from 0d and 13d L*ongissimus* samples stained with coomassie blue.

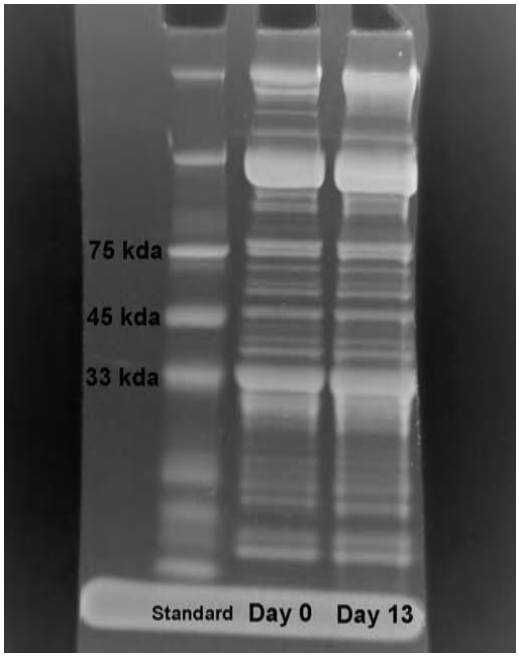

Fig 2. Western Blot with Desmin antibody at 1:500 dilution

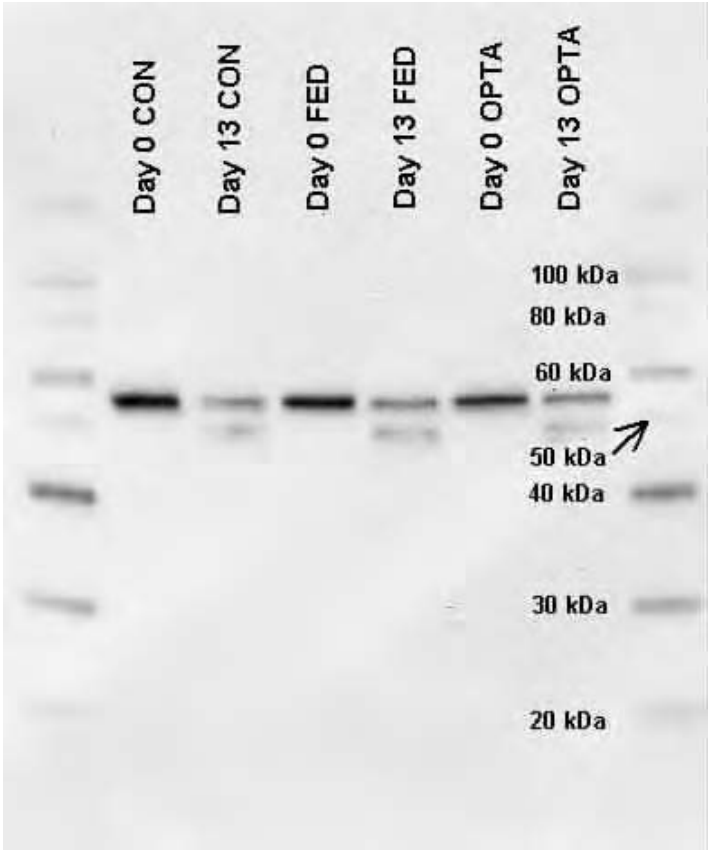

Fig 3. Three-Dimensional image of Desmin Western blot depicting band intensity.

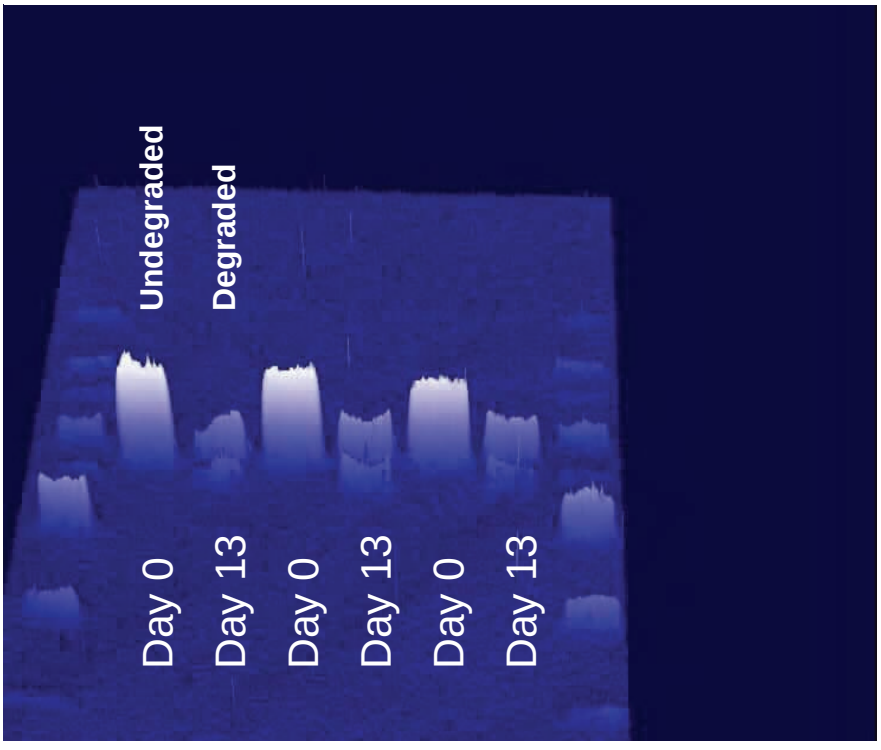

Fig 4. Western Blot with Troponin-T antibody at 1:1,000 dilution

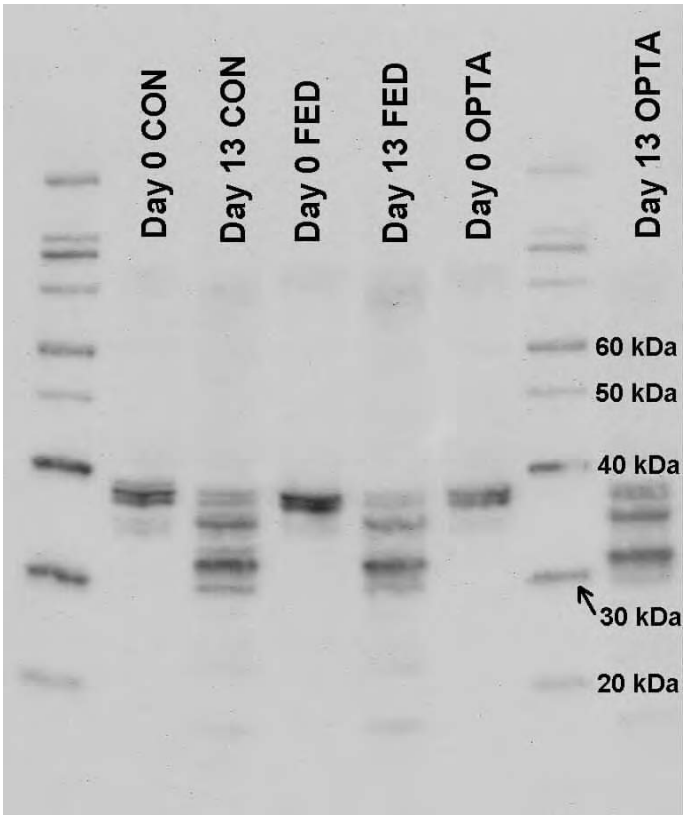

Fig 5. Three-Dimensional image of Troponin-T Western Blot depicting band intensity.