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GROWTH IMPLANTS' EFFECT ON TENDERNESS AND PROTEIN DEGRADATION

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Introduction

Growth implants are routinely used to improve efficiency of meat production by improving red meat yield. Increased feed efficiency and increased *longissimus* muscle area have all been reported with the use of hormonal implants. One problem with this is that there is also a reduction in marbling and therefore fewer numbers of animals grading Choice (Roeber et al., 2000; Platter et al., 2003). Furthermore, these researchers both reported reduced sensory preference for steaks from animals that had been implanted when compared to steaks from animals that had never been implanted.

To accomplish muscle growth, protein accretion must exceed protein breakdown. The enzyme system that is thought to be responsible for controlling protein accretion and breakdown is the calpain system (Boehm et al., 2000). The two parts of the calpain system, μ- and m-calpain, breakdown proteins while the inhibitor, calpastatin, blocks calpain actions. Endogenous growth hormones including testosterone increase the activity of calpastatin and thus increase muscle growth. Uses of exogenous growth hormones such as testosterone in implants increase growth and therefore affect the calpastatin activity. Implant use also increases the proliferation and fusion of satellite cells to increase DNA for protein synthesis (Johnson et al., 1998).

Tenderness of beef has been associated with protein degradation postmortem (Paterson & Parrish, 1986; Huff-Lonergan, 1996). Postmortem protein degradation starts with the calpain system breaking down the cytoskeletal proteins (titin, nebulin, desmin) along with troponin T (Boehm et al., 1998). Some researchers have tried to relate calpain activity to tenderness but have been unsuccessful. The activity of calpastatin at 24 hr, however, has been shown to explain roughly 40% of the tenderness variation in steaks (Whipple et al., 1990; Shackelford et al., 1994).

Recently concern has been raised about aggressive implant strategies reducing tenderness of steaks from treated steers. Roeber and co-workers (2000) reported that steaks from British steers treated with an estradiol benzoate (24 mg) and trenbolone acetate (120 mg) implant followed by no implant had significantly higher Warner-Bratzler shear values than steaks from steers that were never implanted. Furthermore, consumer panelists found steaks from implanted steers were less tender and juicy than steaks from non-implanted steers. Platter et al. (2003) reported that the closer the implant strategy was applied to slaughter that the shear values were more likely to be affected (implanting during the backgrounding phase significantly increased WBS values when

compared to non-implanted animals) however this change was not seen in consumer satisfaction.

Many researchers have reported reduced marbling and increased ribeye area with the use of implants on British based cattle. Platter et al., (2003) utilized steers with various genetic backgrounds, but did not analyze the project to determine if there was a compounding effect on tenderness when implants were administered to animals with a genetic propensity for greater growth. Late maturing, heavily muscled animals already have a larger rate of protein accretion with reduced degradation than earlier maturing light muscled animals. The addition of growth implants may increase the rate of growth and may compound any tenderness problems that may be created by growth implants.

Objectives

The objective of this experiment was to evaluate the effect of growth implants on the rate, extent, and manner of postmortem degradation of protein as well as tenderness of beef cattle.

Methodology

Steers of British (n=14) and Continental (n=17) breed descent were assigned to either implant or nonimplant treatment. A combination implant containing estradiol benzoate (24 mg) and trenbolone acetate (120 mg) was used. After steers and heifers had been on feed for 120 days, they were shipped to a commercial processing facility (8 h travel with 12 h rest) and harvested following normal industry procedures. After 24 h at 4ºC loin sections (7.62 cm) were removed from each carcass. The loin sections were cut into one 3.3 cm steak for tenderness analysis. Small (10g) samples were vacuum packaged and aged for 7, 14 and 21 days for evaluation of protein degradation.

Steaks were thawed at 4°C for 24 hours. Each steak was weighed before and after cooking to determine cook loss. Eight to ten samples (1.27 x 1.27 x 2.54 cm) for shear force evaluation were removed from each steak parallel to the fiber direction. Samples were sheared once perpendicular to the fiber direction with a TMS 30 Food Texturometer fitted with a Warner-Bratzler shear attachment. The average of the samples sheared was used for statistical analysis.

Purified myofibrils were prepared by the procedure of Boles et al., (1992). After purification, myofibrils were washed twice by re-suspension in 10 volumes (wt/vol; of the original ground muscle) of 5 mM Tris-HCl, pH 8.0, and centrifuged at 3,050 x *g* for 10 min at 4ºC (Wang, 1982). The resulting pellet was then re-suspended in four volumes (wt/vol) of 5 mM Tris-HC1, pH *8.0,* and protein content was determined by a modified biuret procedure conducted in the presence of Tris (Robson et al., 1968). For preparation of samples for SDS-PAGE analysis, 1 mL of the protein solution (5.2 mg/mL) was added to 0.2 mL of tracking dye solution (5% [wt/vol SDS, 50% wt/vol sucrose, .05% [wt/vol bromophenol blue, and 50 mM 2-N-morpholinoethanesulfonic acid, pH 6.5) and 0.1 mL of 2-mercaptoethanol and heated at 50°C for 20 min (Paterson and Parrish, 1986).

The SDS-PAGE was performed according to the procedure of Laemmli (1970) with modifications to accommodate separation of proteins with widely different molecular weights. The amount of protein loaded onto each lane of a slab gel (mm) was 15µg. Gels

containing a 4% (wt/vol) acrylamide stacking gel over a 10% (wt/vol) acrylamide separating gel were prepared from a stock solution of 30% (wt/vol) acrylamide (37: 1 ; acrylamide:N,N‘-bis-methylene acrylamide), 0.375 *M* Tris-Ha, pH 8.0, 0.1% (wt/vol) SDS, and 2 mM EDTA. Changes in large-molecular- mass proteins such as titin were monitored by using a 5% (wt/vol) acrylamide (100:l acrylamide N,N’-bis-methylene acrylamide) separating gel containing 0.375 M Tris-HC1, pH 8.0, 0.1% (wt/vol) SDS, and 2 *mM* EDTA, without a stacking gel. Gels were run at 35 mA on BioRad Mini Protean gels (BioRad, San Francisco, CA). Gels were stained overnight in 0.1% (wt/vol) Coomassie brilliant blue R-250 (Sigma Chemical), 10% (vol/vol) glacial acetic acid, 50% (vol/vol) methanol and then were destained in two changes of the same solution, excluding the Coomassie brilliant blue. A molecular weight protein standard (Sigma Chemical) containing rabbit myosin heavy chains (~205 kDa), β-galactosidase (~116 kDa), phosphorylase b (~97 kDa), BSA (~66 kDa, egg albumin (~45 kDa), and carbonic anhydrase (~29 kDa) was used in the 10% gels to aid in identification of proteins.

Individual animals were used as the experimental unit in both studies. The GLM procedure of SAS was used to analyze carcass and tenderness data. Planned comparisons between implant strategy (implant versus no implant) and genetic classifications (high retail product versus high marbling) or implant strategy, sex and growth potential were done.

Results & Discussion

Growth implants used in Continental steers significantly affected shear force values. When implants were used shear force values were significantly higher ($P < 0.01$) for steaks that were from non-implanted steers (Table 1). Implants significantly increased the shear force values of steaks (77.5N with vs. 66.7N without). Continental steers also had a significantly higher shear force value (77.5N) than British steers (65.7N).

Protein degradation was uniform among all samples (Fig. 1). The samples on gels the in Figure one were from Continental cattle with and without implants and Figure 2 shows cytoskeletal (5% gels) protein breakdown of meat from British cattle, with and without implants. No matter what the breed or implant strategy the degradation of proteins was similar. There was no difference in the appearance of the 30,000 dalton component between tough and tender steaks from implanted and non-implanted steers. MacBride and Parrish (1977) reported a relationship between the appearance of a 30,000 dalton component and the tenderness of steaks. Furthermore no difference in the appearance of titin and nebulin (Fig 2) suggest that there were no differences in the breakdown of cytoskeletal proteins. Huff-Lonergan et al. (1995) reported that the rate of conversion of titin from T1 to T2 was slower in less tender samples than in tender samples

Conclusions

Evaluation of protein degradation on SDS-PAGE showed no effect of growth implants on the rate, extent, or manner of protein degradation in either breed or treatment group. No difference in protein degradation suggests no major differences in protein breakdown or calpastatin activity at these times postmortem. Therefore, more information

is needed to establish the cause of the different tenderness values observed with the use of implants.

References

Boehm, M.L., Kendall, T.L., Thompson, V.F. and Goll, D.E. 1998. Changes in calpains and calpastatin during postmortem storage of bovine muscle. Journal of Animal Science. 76, 2415–2434

Boles, J.A., Parrish, Jr., F.C., Huiatt, T.W. and Robson, R. M. 1992. Effect of porcine stress syndrome on the solubility and degradation of myofibrillar/cytoskeletal proteins. Journal of Animal Sciences 70, 454–464.

Huff-Lonergan, E.J., Parrish, Jr., F.C., and Robson, R.M. 1995. Effects of postmortem ageing time, animal age and sex on degradation of titin and nebulin in bovine longissimus muscle. Journal of Animal Science 73, 1064–1073.

Huff-Lonergan, E. Mitsuhashi, T., Beekman, D. D., Parrish, Jr., F. C., Olson, D.G., and Robson, R. M. 1996. Proteolysis of specific muscle structural proteins by m-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. Journal of Animal Science 74, 993–1008

Johnson, B.J., Halstead, N., White, M.E., Hathaway, M.R., DiCostanzo, A. and Dayton, W.R. 1998. Activation state of muscle satellite cells isolated from steers implanted wit ha combined trenbolone acetate and estradiol implant. Journal of Animal Science. 76, 2779–2786.

Laemmli, U. K 1970. Cleavage of structural proteins during the assembly of the head of bacteroiphage T4. Nature 227:680.

Mac Bride, M. A. and Parrish, Jr., F.C. 1977. The 30,000-dalton component of tender bovine longissimus muscle. Journal of Food Science 42, 1627–1629.

Paterson, B.C. and Parrish, Jr., F.C. 1986. A sensory panel and chemical analysis of certain beef chuck muscles. Journal of Food Science 51, 876–879,896.

Platter, W.J., Tatum, J.D., Belk, K.E., Scanga, J.A., and Smith, G.C. 2003. Effects of repetitive use of hormonal implants on beef carcass quality tenderness, and consumer ratings of beef palatability. Journal of Animal Science 81, 984–996.

Robson, R. M., D. E. Goll and M. J. Temple. 1968. Determination of protein in Tris buffer by the biuret reaction. Analytical Biochemistry 24, 339–341.

Roeber, D.L., Cannell, R.C., Belk, K.E., Miller, R.K., Tatum, J.D. and Smith, G.C. 2000. Implant strategies during feeding: Impact on carcass grades and consumer acceptability. Journal of Animal Science. 78, 1867–1874.

Shackelford, S.D., M. Koohmaraie, L.V. Cundiff, K.E. Gregory, G. A. Rohrer, and J.W. Savell. 1994. Heritabilities and phenotypic and genetic correlations for bovine postrigor calpastatin activity, intramuscular fat content, Warner-Bratzler shear force, retail product yield, and growth rate. Journal of Animal Science 72:857–863.

Wang, K. 1982. Purification of titin and nebulin. Methods in Enzymology 85, 264

Whipple, G., M. Koohmaraie, M.E. Dikeman, and J. D. Crouse. 1990. Predicting beef-longissimus tenderness from various biochemical and histological muscle traits. Journal of Animal Science 68:4193–4199.

Table 1**: Effect of breed type and growth implants on tenderness.**

			Shear force, N
Breed type[a]		Continental	77.5
		British	65.7
		P – value	<0.01
Implant		With	77.5
		Without	66.7
		P – value	<0.01
Breed Implant	×	Continental with	85.3
		British with	68.6
		Continental without	69.6
		British without	62.8
		P – value	0.26

[a]Breed types were characterized by what the sire was known to be. Continental descent cattle were from Simmental sires whereas British descent cattle were from Angus and Hereford sires.

Figure 1: Ten percent SDS-PAGE of isolated myofibrils from normal and implanted steers at 7, 14, and 21 days. 15µg of total protein was loaded onto each lane. Number to the left indicates the protein molecular weights in kDa. Sample 515 was a Continental implanted, 593 was a Continental non-implanted steer, 629 was a Continental implanted steer, and 607 was a Continental non-implanted steer.

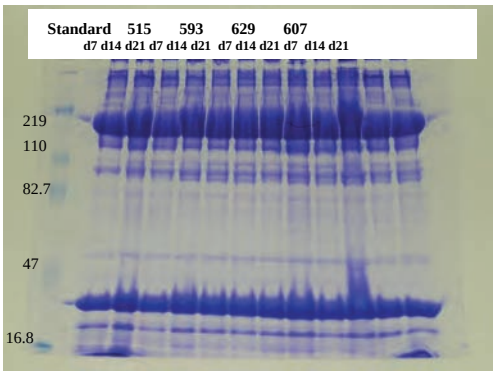

Figure 2: Five percent SDS-PAGE of isolated myofibrils from normal and implanted steers at 7, 14, and 21days. 15µg of total protein was loaded onto each lane. Sample 571 was a British non-implanted steer, 495 was a British non-implanted steer, 553 was a British implanted steer, and 561 was a British implanted steer.