Protein turnover and sensory traits of longissimus muscle from implanted and nonimplanted heifers[1]

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ABSTRACT: Primary bovine muscle cell culture studies were conducted to determine whether implanting heifers had a direct effect on in vitro protein synthesis and degradation and to determine the effect of implanting heifers on longissimus muscle palatability. Feedlot heifers (n = 96) were administered one of six implant regimens to characterize their effect on in vitro amino acid uptake and protein degradation. Treatments consisted of: 1) a nonimplanted control (NI/NI); 2) no implant on d 1 and Revalor-H administered on d 84 of the experiment (NI/Rev); 3) Revalor-H on d 1, but no implant given at d 84 (Rev/NI); 4) Revalor-H administered on d 1 and d 84 (Rev/Rev); 5) Revalor-IH administered on d 1 and Revalor-H at d 84 (RIH/Rev); and 6) Synovex-H given at d 1 and Revalor-H administered at d 84 (Syn/Rev). Blood and longissimus lumborum muscle were collected 20 min postmortem, and serum and muscle extracts were incubated with primary bovine muscle cells. Implant treatments had minimal effects on shear force and sensory traits; however, steaks from Rev/Rev heifers were 0.31 kg more tender ($P < 0.05$) than steaks from NI/NI heifers. Serum protein synthesis and degradation were not affected ($P > 0.10$) by any implant treatment. When primary bovine muscle cells were treated with muscle extract, amino acid uptake was greater for heifers implanted with Rev/Rev than for the average of all other treatments ($P < 0.01$). The Rev/Rev implant regimen also increased ($P < 0.05$) amino acid uptake compared with heifers treated with RIH/Rev, Syn/Rev, NI/NI, NI/Rev, or Rev/NI. Cellular protein degradation of the muscle cell culture treated with muscle extract tended ($P < 0.10$) to be higher in NI/NI-treated cells compared with the average of all implant treatments. In addition, cells treated with muscle extract from heifers implanted with Rev/Rev had lower ($P < 0.05$) protein degradation than the NI/NI control heifers. These results indicate that anabolic implant strategies can directly affect both muscle protein synthesis and degradation via effects that seem to be more autocrine than paracrine in nature.

Key Words: Anabolic Agents, Bovine, Muscle Cells, Protein Turnover

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Introduction

Anabolic implants are used extensively by the feedlot industry to promote growth and increase the efficiency of protein deposition (Trenkle, 1976; Roeder et al., 1986). Estrogens and androgens are used to increase muscle protein accretion and improve efficiency by increasing protein synthesis and/or decreasing protein degradation. Although the mechanisms by which these agents increase muscle growth are uncertain, they are known to have direct effects on protein synthesis and/or degradation in the muscle cell or modify hormone concentrations, which in turn produce the anabolic effects in muscle (Buttery, 1978; Flaim et al., 1978; Roeder et al., 1986).

Anabolic agents have been shown to increase satellite cell number, water concentration, and N content (Vernon and Buttery, 1978; Ranaweera and Wise, 1981; Johnson et al., 1998). Previous studies (Duckett et al.; 1997; Morgan, 1997) have shown that implants can sometimes have negative effects on palatability traits. Currently, a variety of implant programs are used by the industry. Little information is known about how different implant programs affect muscle cell protein synthesis and degradation and palatability traits. The experimental objectives were to characterize various

[1]Names are necessary to report factually on available data; however, Auburn University, Texas Tech University, and the Texas Tech Health Science Center do not guarantee or warrant the standard of the product, and the use of the name implies no approval of the product to the exclusion of others that might also be suitable.

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implant programs with regard to their effects on muscle protein synthesis and degradation in a primary bovine muscle cell culture system and on palatability traits of longissimus steaks.

Materials and Methods

Animals

Two hundred eighty-eight medium-framed beef heifers (Charolais sires mated to Angus- or Hereford-crossbred dams [dams were 25 to 37.5% Brahman]) were shipped to the Texas Tech University Burnett Center. Forty-eight pens of six heifers were assigned randomly to one of six implant treatments consisting of: 1) a nonimplanted control (**NI/NI**); 2) no implant on d 1 and Revalor-H administered on d 84 of the experiment (**NI/Rev**); 3) Revalor-H on d 1, but no implant given at d 84 (**Rev/NI**); 4) Revalor-H administered on d 1 and d 84 (**Rev/Rev**); 5) Revalor-IH (a new implant pending FDA approval contains 80 mg of trenbolone acetate and 8 mg of estradiol) administered on d 1 and Revalor-H at d 84 (**RIH/Rev**); and 6) Synovex-H given at d 1 and Revalor-H administered at d 84 (**Syn/Rev**).

Heifers were fed a 90% concentrate (steam-flaked corn-based) diet with supplemental vitamin/mineral premix and added Rumensin (33 mg/kg, DM basis) and Tylan (8.8 mg/kg, DM basis; Galyean et al., 1999). All heifers were shipped to a commercial packing plant when average pen weights reached approximately 500 kg. The heifers were slaughtered and USDA quality and yield grade factors (USDA, 1989) were collected after chilling (2°C spray-chill) for 48 h. Of the 279 heifers that were shipped to the packing plant, 96 (eight heifers in six treatments randomly selected from within two kill groups) were subsampled to simplify collection of samples for muscle extract culture analysis.

Fabrication

At 48 h postmortem, a boneless strip loin (Institutional Meat Purchasing Specifications [**IMPS**] #180; USDA, 1990) was removed from each carcass, labeled, vacuum packaged, and transported to Texas Tech University for aging (4°C). Boneless, 2.5-cm-thick strip loin steaks were fabricated at 14 d postmortem. The steaks then were vacuum packaged and frozen at –20°C for about 30 d until Warner-Bratzler shear force (**WBSF**) and trained sensory panel analyses were conducted.

Myofibrillar Fragmentation Index

At 14 d postmortem, a 4-g sample was removed from the longissimus muscle of each strip loin for determination of myofibrillar fragmentation index (**MFI**). The procedures of Culler et al. (1978) were followed for MFI measurement.

Cell Culture

Because of the concern of relevance of results from cloned murine myoblasts to ruminant domestic livestock, bovine myoblasts were used in cell culture assays. Primary bovine myoblast cultures were prepared following procedures outlined by Hembree et al. (1991). A first-trimester fetus (approximately 55 d postconception) was placed in a sterile field, and the skin was removed from the hind limb. Small pieces of muscle tissue were removed prerigor and placed in a vial of cold, sterile-filtered RPMI-1640 (RPMI, Sigma-Aldrich Co., St. Louis, MO) culture medium (without L-glutamine), containing 10% (vol/vol) fetal bovine serum (**FBS**). The samples then were transported (4°C) to the laboratory where the muscle was minced with a razor blade and transferred to a vial containing RPMI with 10% (vol/vol) FBS and 5% (wt/vol) collagenase. Samples were stirred constantly for 1.5 h at room temperature. The upper portion of the culture was transferred to a centrifuge tube and centrifuged for 10 min at 1,000 × *g*. The collagenase medium was removed and replaced with fresh RPMI plus 10% (vol/vol) FBS and resuspended by vortexing. The cells were transferred to 75-cm^2 culture flasks and incubated at 37°C with 100% humidity, 5% CO_2, and 95% air. The cells were allowed to grow for 48 to 72 h, and then trypsinized to release them from the flask, transferred to a 15-mL conical tube, and centrifuged (1,000 × *g* for 5 min). Trypsin medium was aspirated, and cells were resuspended in fresh RPMI buffer with 10% (vol/vol) FBS. To decrease the inclusion of fibroblasts in the cell culture, cells were added to a 75-cm^2 culture flask and incubated (37°C) for 30 min. The medium (containing unattached myoblasts) was then transferred to a new culture flask. Because fibroblasts attach to the flask more quickly than myoblasts, this preplating procedure has been useful for obtaining a relatively pure primary myoblast culture.

Serum Preparation

To prepare the serum for the cell culture assays, blood from each animal was collected during exsanguination and held at room temperature (25°C) for approximately 6 h. Each tube then was centrifuged (1,000 × *g* for 10 min) to separate the clot from the serum. Serum from each animal was added to skeletal muscle basal medium (**SkBM**, Clonetics Inc., San Diego, CA; 5% vol/vol). The serum/medium mixture was then sterilized by filtration through a 0.22-μm filter.

Muscle Extract Preparation

Muscle extract was prepared following the procedures of Haugk et al. (1995) with the modifications described by Kerth (1999). A 10-g sample of longissimus muscle was removed from the left side of the carcass (20 min after exsanguination [prerigor]) for muscle extraction. All visible fat and connective tissue were removed, and the sample was placed in a 50-mL conical tube with 30 mL of extraction buffer (50 m*M* Tris and 10 m*M* EDTA with leupeptin, ovomucoid, and phenyl-

Table 1. Content and radioactivity of amino acid mixture used for labeling proteins in vitro

Amino acid	Specific activity, mCi/mmol
Alanine	152
Arginine	270
Aspartic acid	200
Glutamic acid	257
Glycine	103
Histidine	337
Isoleucine	321
Leucine	320
Lysine	322
Phenylalanine	531
Proline	279
Serine	162
Threonine	199
Tyrosine	507
Valine	225

methylsulfonyl fluoride to inhibit proteolysis). The sample was homogenized for 45 s using a tissue tearer and transferred to a 50-mL high-speed centrifuge tube. The samples were centrifuged (40,000 × *g* for 60 min) and filtered through cheesecloth to clarify. Protein concentration of each sample was determined with the biuret assay using known BSA concentrations of 0, 2.5, 5.0, 7.5, and 10.0 mg/mL of protein (Layne, 1957), and 400 μg/mL of muscle protein and 3% (wt/vol) FBS was added to SkBM and filtered through a 0.22-μm filter.

Protein Synthesis Assay

The procedure for determining the rate of protein synthesis as measured by uptake of labeled amino acids was conducted as described by Reecy et al. (1994). Briefly, bovine myoblasts were plated in 24-well plates (approximately 5,000 cells/well determined using hemocytometry) and allowed to grow for 48 h (to reach 90% confluency). The medium then was aspirated and replaced with 1.0 mL of medium containing the treatment serum or muscle extract. After a 24-h incubation, 1 μCi of a ^{14}C-labeled amino acid mixture (Table 1) was added to each well. The cells were labeled for 2 h, the medium was removed, and then the cells were lysed by adding 0.5 mL of 1 *M* NaOH to each well. After 2 h, 0.5 mL of 20% (wt/vol) TCA (trichloroacetic acid) was added and the plates were placed in the refrigerator overnight. Cells then were harvested, transferred to a 2.5-cm-diameter glass fiber filter disc, dried, and transferred to a liquid scintillation vial. Five milliliters of a liquid scintillation counting cocktail was added, and activity was measured as disintegrations per minute (dpm) in a Beckman liquid scintillation counter (model 6500, Fullerton, CA). Amino acid uptake was defined as dpm of the cell layer. Serum or muscle extract from each animal served as the treatment and was added to the SkBM at 5% (vol/vol). All assays were performed in triplicate.

Protein Degradation Assay

Muscle cell protein degradation percentage was determined by following procedures described by Ballard et al. (1986). Bovine myoblasts were plated in 24-well plates (approximately 5,000 cells/well determined by hemocytometry) and allowed to grow for 48 h. The medium was replaced by 1.0 mL of SkBM with 10% (vol/vol) FBS and 1 μCi of ^{14}C-labeled amino acids (Table 1). After a 24-h incubation (37°C), the labeling medium was removed and each well was washed twice with fresh medium. One milliliter of SkBM with treatment serum or muscle extract (chase medium) was placed in each well and incubated for 4 h. The chase medium was removed, each well was rinsed, and 1.0 mL of SkBM containing 5% (vol/vol) treatment serum or muscle extract (using SkBM only, as a control) was placed in each well. Each plate then was incubated for an additional 2 h. One-half milliliter of the medium was transferred to a 1.5-mL tube, and 0.5 mL of cold 20% TCA ([vol/vol] final concentration of 10% TCA) was added, vortexed, transferred to a glass-fiber filter, and rinsed with 5% (vol/vol) TCA. Additionally, 0.5 mL of the medium was transferred to a scintillation vial and counted for total medium dpm. Finally, the cells were lysed, harvested, and counted as described for amino acid uptake. The samples for each animal were analyzed in triplicate. Protein degradation was expressed as follows:

$$\text{Protein degradation, \%} = \frac{\text{dpm of TCA precipitate} \times 100}{\text{dpm of total medium + dpm of cell layer}}$$

Warner-Bratzler Shear Force

Steaks were thawed for 24 h at 4°C and then cooked on open-hearth broilers (Farberware, Bronx, NY) to 40°C, turned, and removed when they reached 71°C (AMSA, 1995). Internal temperature was monitored with a stainless steel puncture probe connected to a Cooper Instruments digital thermometer (model SH66A, Middlefield CT). The probe was placed in the center of each steak. Steaks were then cooled at 4°C for 24 h, and six 1.3-cm diameter cores were removed from each steak parallel to the orientation of the muscle fibers. Each core was sheared once perpendicular to the muscle fiber with a WBSF instrument (GR Electric Mfg., Manhattan, KS).

Trained Sensory Panel

Steaks were thawed and cooked as described for WBSF measurements. After cooking, fat and connective tissue were removed and each steak was cut into cubes (1 cm × 1 cm × steak thickness) and placed in pans kept warm with heated sand. Two cubes were served to each member of an eight-member trained sensory panel

Table 2. Overall means, standard deviations, and range for heifer carcass data

Trait	N	Mean	SD	Minimum	Maximum
Live weight, kg	92	510.0	43.7	405.0	611.8
Hot carcass weight, kg	91	319.3	30.1	253.6	385.0
Dressing percentage	91	62.6	1.80	57.6	68.7
Marbling[a]	92	408.8	91.0	220.0	730.0
Quality grade[b]	92	11.2	0.81	8.0	15.0
Preliminary yield grade	92	2.96	0.44	2.20	4.70
Adjusted preliminary yield grade	92	3.23	0.40	2.60	4.50
Kidney, pelvic, and heart fat, %	92	2.51	0.71	1.50	5.00
Longissimus area, cm^2	92	89.33	8.97	69.66	116.1
USDA yield grade	91	2.59	0.72	1.07	4.92

[a]200 = practically devoid^{00}, 400 = slight^{00}, 700 = moderate^{00}.
[b]8 = Standard^{-}, 11 = Select^{-}, 15 = Choice^{+}.

(Cross et al., 1978). Panel members were served samples under red lighting and were supplied with apple juice and water for rinsing the palate and a cup for expectoration. Members evaluated each sample on a scale of 1 to 8 for initial juiciness, sustained juiciness, initial tenderness, sustained tenderness, flavor intensity, beef flavor, and overall mouth feel (1 = extremely dry, tough, bland, off-flavor, and uncharacteristic of young beef; 8 = extremely juicy, tender, intense, beef-like and characteristic of young beef; AMSA, 1995).

Experimental Design

Treatments were arranged in a randomized complete block design with the two kill groups serving as blocks. Data were analyzed using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC), with block and implant regimen in the model and the residual variance serving as the error term. Meaningful comparisons among implant treatments were sought and means were analyzed using nonorthogonal, linear contrasts to compare the various implant combinations: NI/NI vs. all other implants, NI/NI vs. all double implanted, NI/NI vs. all single implants, single implant vs. double implant, each implant vs. NI/NI, each double implant vs. each double implant, and NI/Rev vs. Rev/NI were compared. Simple correlations were calculated using the CORR procedure in SAS. The acceptable level of making a type-I error was 5%.

Results

Carcass Data

Heifers used to determine the protein synthesis/degradation assays and sensory evaluation averaged 510 kg at slaughter and had an average dressing percentage of 62.6% (Table 2). Marbling score averaged low Slight (408.8), which resulted in an average low Select USDA quality grade. The preliminary yield grade and adjusted preliminary yield grade of 2.96 and 3.23, respectively, indicated carcasses had about 1.0 cm of fat at the 12th rib. The average final yield grade was 2.6, but the carcasses ranged from yield grade 1.1 to 4.9. Performance and carcass data for the 279 heifers used in the overall experiment were previously reported by Galyean et al. (1999).

Myofibrillar Degradation, Warner-Bratzler Shear Force, and Sensory Traits

Means and standard errors for MFI, WBSF, cooking loss, and sensory traits are presented in Table 3. Linear contrasts indicated that total MFI, cooking loss, initial juiciness, sustained tenderness, beef flavor, and overall mouth feel traits were not affected ($P > 0.10$) by implant regimen. Mean WBSF values were greater ($P < 0.05$) for steaks from NI/NI heifers compared with steaks from heifers implanted with Rev/Rev, but no other implant regimen differed ($P > 0.10$).

Sustained juiciness scores of steaks from heifers implanted with NI/Rev were higher than steaks from heifers implanted with Rev/Rev ($P < 0.05$) and tended to be higher than heifers implanted with RIH/Rev ($P < 0.10$). Sustained juiciness scores were not affected ($P > 0.10$) by any other implant or implant combination treatment. Steaks from heifers implanted with Rev/NI tended to have higher ($P < 0.10$) initial tenderness and flavor intensity scores compared with steaks from heifers implanted with Syn/Rev. All other implant combination treatments did not affect ($P > 0.10$) initial tenderness, flavor intensity, or overall mouth feel.

Cellular Amino Acid Uptake and Protein Degradation

Amino acid uptake and protein degradation measured by treating fetal muscle cells with serum from heifers were not affected ($P > 0.10$) by any implant combination; however, when fetal muscle cells were treated with muscle extract, amino acid uptake was greater ($P < 0.01$) for muscle extract from heifers implanted with Rev/Rev than for all other implant treatments (Table 4). Extract from heifers implanted with Rev/Rev also had higher ($P < 0.05$) amino acid uptake than that from heifers receiving NI/NI, NI/Rev, Rev/NI, RIH/Rev, or Syn/Rev. Amino acid uptake was not affected ($P > 0.10$) by any other implant treatment combination. Cellular protein degradation tended ($P < 0.10$)

Table 3. Means and standard errors for MFI, WBSF, and sensory traits of heifers receiving six different implant regimens

Trait	First implant/ second implant: No implant No implant	No implant Revalor	Revalor No implant	Revalor Revalor	Revalor-IH Revalor	Synovex Revalor	SEM
MFI[a]	67.5	68.9	68.8	66.1	64.8	67.1	1.93
Shear force, kg[x]	3.49	3.54	2.93	3.18	3.34	3.39	0.202
Cooking loss, %	17.1	17.9	17.7	17.3	16.5	17.5	0.93
Initial juiciness[b]	6.1	6.3	6.1	6.1	6.2	6.2	0.12
Sustained juiciness[by]	6.3	6.5	6.3	6.1	6.2	6.3	0.12
Initial tenderness[b]	5.7	5.9	6.1	6.0	5.8	5.6	0.22
Sustained tenderness[b]	5.8	6.0	6.0	6.1	5.9	5.6	0.21
Flavor intensity[b]	6.2	6.2	6.3	6.2	6.2	6.0	0.10
Beef flavor[b]	6.3	6.4	6.4	6.3	6.4	6.3	0.11
Overall mouth feel[b]	5.6	5.9	5.9	5.9	5.8	5.5	0.19

[a]Myofibrillar fragmentation index.
[b]5 = slightly juicy, juicy, tender, tender, intense, characteristic beef flavor, or characteristic of young beef, 6 = moderately juicy, juicy, tender, tender, intense, characteristic beef flavor, or characteristic of young beef.
[x]Revalor/Revalor treatment was different ($P < 0.05$) from the no implant/no implant treatment.
[y]Revalor/Revalor treatment was different ($P < 0.05$) from the no implant/Revalor treatment.

to be greater in NI/NI-treated cells compared to implant treatments. In addition, cells treated with extract from heifers implanted with Rev/Rev had 6.3% less ($P < 0.05$) protein degradation than cells treated with extract from the NI/NI control heifers.

Correlations

Myofibrillar fragmentation index was related negatively to WBSF and positively to initial tenderness, sustained tenderness, and overall mouth feel ($P < 0.05$, Table 5). The MFI, serum uptake and degradation, and muscle extract uptake and degradation were not correlated ($P > 0.05$) with any other sensory trait or WBSF, nor were they correlated with any carcass trait ($P > 0.05$; data not shown).

Discussion

Implanting beef cattle promotes growth and increases the efficiency of protein deposition (Trenkle, 1976; Roeder et al., 1986). Use of anabolic agents, such as trenbolone acetate, has been shown to improve muscling and carcass yield (Apple et al., 1991; Johnson et al., 1996). Although anabolic implants improve production efficiency of beef cattle, the effects of implants on tenderness have produced mixed results. Some studies have shown that implanting beef cattle decreased tenderness and increased WBSF (Samber et al., 1996; Duckett et al., 1997; Morgan, 1997), whereas others have found that some implant regimens have no effect on tenderness or WBSF (Gerken et al., 1995; Pruneda et al., 1999). The present study showed no adverse ef-

Table 4. Means and standard errors for cellular amino acid uptake and protein degradation in muscle cells treated with either muscle extract or serum from heifers administered six different implant regimens

Trait	First implant/ second implant: No implant No implant	No implant Revalor	Revalor No implant	Revalor Revalor	Revalor-IH Revalor	Synovex Revalor	SEM
Serum							
Amino acid uptake[a]	1,314.0	1,222.8	1,286.7	1,179.1	1,468.2	1,312.4	147.81
Protein degradation[b]	29.3	28.8	25.3	29.5	17.8	25.7	7.14
Muscle extract							
Amino acid uptake[ax]	550.3	564.6	525.5	755.8	608.2	518.4	110.09
Protein degradation[by]	16.8	12.0	12.3	10.5	13.8	16.0	2.68

[a]Amino acid uptake = dpm of cell layer after a 2-h incubation with treatment and ^{14}C-labeled amino acids.
[b]Protein degradation, % = (dpm of TCA precipitate × 100)/(dpm of total medium + dpm of cell layer).
[x]Revalor/Revalor treatment was different ($P < 0.05$) from all other implant treatments.
[y]Revalor/Revalor treatment was different ($P < 0.05$) from the no implant/no implant treatment.

Table 5. Simple correlation coefficients for muscle protein turnover measurements, Warner Bratzler shear force (WBSF), and sensory traits

Traits	Serum uptake	Muscle uptake	Serum degradation	Muscle degradation	WBSF, kg	Initial tenderness	Sustained tenderness	Overall mouth feel
Myofibrillar fragmentation index	0.08	–0.12	0.05	–0.01	–0.46*	0.37*	0.37*	0.25*
Serum uptake	—	0.47*	–0.65*	0.11	0.08	–0.07	–0.06	–0.06
Muscle uptake	—	—	–0.69*	0.17	0.08	–0.03	0.01	0.04
Serum degradation	—	—	—	–0.18	–0.09	0.12	0.11	0.07
Muscle degradation	—	—	—	—	0.15	0.06	0.02	0.09
WBSF, kg	—	—	—	—	—	–0.71*	–0.71*	–0.63*
Initial tenderness	—	—	—	—	—	—	0.94*	0.91*
Sustained tenderness	—	—	—	—	—	—	—	0.91*

*$P < 0.05$.

fects of implants or hormone concentration on WBSF or palatability traits in heifers that were implanted with any combination of implant treatments.

Because steaks from implanted cattle sometimes tend to be less tender, we hypothesized that the mechanism for increased muscling in implanted cattle is caused by a decrease in protein degradation. This decreased degradation antemortem was then thought to reduce the effect of aging on postmortem proteolysis/tenderization. Results from the present study support this theory as shown by the decrease in vitro protein degradation for primary bovine muscle cells treated with muscle extracts from implanted heifers compared with those from NI/NI heifers, whereas heifers from the Rev/Rev treatment had lower shear force values, which refutes the theory. The present study also indicates that muscle protein accretion is enhanced (increased amino acid uptake in Rev/Rev heifers), inferring that protein synthesis is increased by the Rev/Rev treatment, which refutes the notion that implants act primarily by reducing protein degradation. Most likely, the increase in muscling associated with implanting beef cattle is the net result of increased protein synthesis and decreased protein degradation.

Protein turnover in muscle is a ratio of muscle protein accretion and muscle cell proliferation to protein degradation. A variety of methods, including N^{τ}-methylhistidine excretion, infusion with [$^2H_5$]-phenylalanine, and radioactively labeling live animals, have been used to measure protein synthesis and degradation (Jones et al., 1986; Hayden et al., 1992; Lorenzen et al., 2000). These methods can be both expensive and labor intensive. The method used in the present study for measuring skeletal muscle protein turnover is less labor intensive and is not as controversial as the use of radioactive materials in live animals.

Implant regimen did not affect cellular protein synthesis or degradation differences in the present study when serum was added to the cell cultures. Skjaerlund et al. (1988) also demonstrated that protein turnover was not affected by adding serum to skeletal muscle strips, whereas Thomson et al. (1996) found that incubating bovine fetal muscle cells with serum from steers that had been treated with bovine somatotropin or steroidal implants increased amino acid uptake, but did not affect protein degradation. Johnson et al. (1996) reported that adding serum from steers treated with trenbolone acetate and estradiol implants increased proliferation of muscle satellite cells. Because protein turnover differed in muscle extracts depending on implant type and not in serum-treated cultures, it is possible that implants have an effect on muscle growth that is autocrine, rather than paracrine, in nature. Anabolic agents have a variety of effects on muscle at the cellular level. Androgen effects on muscle growth have been linked to changes in insulin, IGF-1, and antogonistic effects on the normal catabolic action of glucocorticoids (Florini, 1987; Celotti and Cesi, 1992; Rooyackers and Nair, 1997). Understanding anabolic steroid effects on muscle protein turnover is further complicated by the fact that sarcoplasmic and myobibrillar protein degradation pathways function independently of each other and involve multiple pathways (Fernandez and Sainz, 1997).

In the present study, administration of a trenbolone acetate/estradiol combination implant increased protein synthesis and decreased protein degradation. Trenbolone acetate and estradiol implants have been shown to increase circulating IGF-1 and IGFBP concentrations and to elevate satellite cell numbers (Johnson et al., 1996, 1998). Johnson et al. (1996) reported that IGF-1 concentrations decreased over the feeding period, and the observed decrease in IGF-1 concentration might explain the lack of implant effects when serum samples taken at harvest were added to cultures. The binding capacity of the testosterone receptor on skeletal muscle has been shown to decrease over time when exposed to trenbolone acetate (Sinnett-Smith et al., 1987); thus, if serum samples had been taken earlier in our experiment, we might have seen a larger paracrine effect on protein turnover.

Results of the present study indicate that fetal bovine muscle cells treated with muscle extracts could be used to detect changes in protein degradation and amino acid uptake in vitro, and the method does not require as many resources as traditional indirect detection

methods. Treatment of muscle cells with serum from implanted animals did not allow for detection of changes in protein turnover for implanted vs. nonimplanted beef cattle in the present study. Trenbolone acetate and estradiol implantation have been shown to increase skeletal muscle protein, N-accretion, and intracellular water content (Vernon and Buttery, 1978; Ranaweera and Wise, 1981; Perry et al., 1991). Unfortunately, results on the effects of anabolic steroids on cellular protein synthesis and degradation are contradictory. Testosterone and estradiol were shown to have no effect on muscle cellular protein synthesis or degradation (Roeder et al., 1986; Desler et al., 1996), whereas Martinez et al. (1984) and Hayden et al. (1992) showed that testosterone and trenbolone acetate increased protein synthesis only. Conversely, Hunter and Magner (1990) found trenbolone acetate decreased protein degradation. In general, our results agree with the body of literature that trenbolone acetate had its strongest effect on protein synthesis and not protein degradation.

Implications

It appears that implants trigger a mechanism within the muscle cell that increases protein accretion rather than affecting a regulatory mechanism circulating in the blood. This mechanism affects both protein synthesis and protein degradation as opposed to mainly decreasing protein degradation, as was previously thought. As a result, implants may not necessarily decrease beef tenderness.

Literature Cited

AMSA. 1995. Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Fresh Meat. Am. Meat Sci. Assoc., Chicago, IL.

Apple, J. K., M. E. Dikeman, D. D. Simms, and G. Kuhl. 1991. Effects of synthetic hormone implants, singularly or in combinations, on performance, carcass traits, and longissimus muscle palatability of Holstein steers. J. Anim. Sci. 69:4437–4448.

Ballard, F. J., L. C. Read, G. L. Francis, C. J. Bagley, and J. C. Wallace. 1986. Binding properties and biological potencies of insulin-like growth factors in L6 myoblasts. Biochem. J. 233:223–230.

Buttery, P. J., B. G. Vernon, and J. T. Pearson. 1978. Anabolic agents—Some thoughts on their mode of action. Proc. Nutr. Soc. 37:311–315.

Celotti, F., and P. N. Cesi. 1992. Anabolic steroids: A review of their effects on the muscles, of their possible mechanisms of action and of their use in athletics. J. Steroid Biochem. Mol. Biol. 43:469–477.

Cross, H. R., R. Moen, and M. S. Stanfield. 1978. Training and testing of judges for sensory analysis of meat quality. Food Technol. 37:48–54.

Culler, R. D., J. F. C. Parrish, G. C. Smith, and H. R. Cross. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43:1177–1180.

Desler, M. M., S. J. Jones, C. W. Smith, and T. L. Woods. 1996. Effects of dexamethasone and anabolic agents on proliferation and protein synthesis and degradation in C_2C_{12} myogenic cells. J. Anim. Sci. 74:1265–1273.

Duckett, S. K., F. N. Owens, and J. G. Andrae. 1997. Effects of implants on performance and carcass traits of feedlot steers and heifers. Pages 63–82 in Proc. Symp. Impact of Implants on Performance and Carcass Value of Beef Cattle, Oklahoma Agric. Exp. Stn., P-957.

Fernandez, C., and R. D. Sainz. 1997. Pathways of protein degradation in L6 myotubes. Proc. Soc. Exp. Biol. Med. 214:242–247.

Flaim, K. E., J. B. Li, and L. S. Jefferson. 1978. Effects of thyroxine on protein turnover in rat skeletal muscle. Amer. J. Physiol. 235:231–237.

Florini, J. R. 1987. Hormonal control of muscle growth. Muscle Nerve 10:577–598.

Galyean, M. L., S. C. Harris, H. M. Derington, P. F. Defoor, G. A. Nunnery, and G. B. Salyer. 1999. Effects of various implant programs on performance and carcass merit of finishing heifers. Burnett Center Internet Prog. Re. No. 4—October, 1999. Texas Tech Univ., Lubbock.

Gerken, C. L., J. D. Tatum, J. B. Morgan, and G. C. Smith. 1995. Use of genetically identical (clone) steers to determine the effects of estrogenic and androgenic implants on beef quality and palatability characteristics. J. Anim. Sci. 73:3317–3324.

Haugk, K. L., R. A. Roeder, M. J. Garber, and G. T. Schelling. 1995. Regulation of muscle cell proliferation by extracts from crushed muscle. J. Anim. Sci. 73:1972–1981.

Hayden, J. M., W. G. Bergen, and R. A. Merkel. 1992. Skeletal muscle protein metabolism and serum growth hormone, insulin, and cortisol concentrations in growing steers implanted with estradiol-17β, trenbolone acetate, or estradiol-17β plus Trenbolone acetate. J. Anim. Sci. 70:2109–2119.

Hembree, J. R., M. R. Hathaway, and W. R. Dayton. 1991. Isolation and culture of fetal porcine myogenic cells and the effect of insulin, IGF-1, and sera on protein turnover in porcine myotube cultures. J. Anim. Sci. 69:3241–3250.

Hunter, R. A., and T. Magner. 1990. Whole-body and tissue protein synthesis in steers losing weight on a low-protein roughage diet: the effect of trenbolone acetate. J. Agric. Sci. (Cambridge) 115:121–127.

Johnson, B. J., P. T. Anderson, J. C Meiske, W. R. Dayton. 1996. Effect of a combined trenbolone acetate and estradiol implant on feedlot performance, carcass characteristics and carcass composition of feedlot steers. J. Anim. Sci. 74:363–371.

Johnson, B. J., N. Halstead, M. E. White, M. R. Hathaway, A. DiCostanxo, and W. R. Dayton. 1998. Activation of muscle satellite cells ilolated from steers implanted with a combined trenbolone acetate and estradiol implant. J. Anim. Sci. 76:2779–2786.

Jones, S. J., E. D. Aberle, and M. D. Judge. 1986. Skeletal muscle protein turnover in broiler and layer chicks. J. Anim. Sci. 62:1576–1583.

Kerth, C. R. 1999. Characterizing muscle growth and meat quality in callipyge sheep using a muscle cell culture system. Ph.D. Diss., Texas Tech. Univ., Lubbock.

Layne, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. Page 450 in Methods in Enzymology. S. P. Colowick and N. O. Kaplan, ed. Vol. 13. Academic Press, Inc., New York.

Lemus, A. E., F. Vilchis, R. Damsky, B. A. Chavez, G. A. Garcia, I. Grillasca, and G. Perez-Palacios. 1992. Mechanism of action of levonorgestrel: In vitro metabolism and specific interactions with steroid receptors in target organs. J. Steroid Biochem. Mol. Biol. 41:881–890.

Lorenzen, C. L., M. Koohmaraie, S. D. Shackelford, F. Jahoor, H. C. Freetly, T. L. Wheeler, J. W. Savell, and M. L. Fiorotto. 2000. Protein kinetics in callipyge lambs. J. Anim. Sci. 78:78–87.

Martinez, J. A., P. J. Buttery, and J. T. Pearson. 1984. The mode of action of anabolic agents: The effect of testosterone on muscle protein metabolism in the female rat. Br. J. Nutr. 52:515–521.

Morgan, J. B. 1997. Implant program effects on USDA beef carcass quality grade traits and meat tenderness. Pages 147–154 in Symp. Impact of Implants on Performance and Carcass Value of Beef Cattle. Oklahoma Agric. Exp. Stn., P-957.

Perry, T. C., D. G. Fox, and D. H. Beerman. 1991. Effect of an implant of trenbolone acetate and estradiol on growth, feed efficiency, and carcass composition of holstein and beef steers. J. Anim. Sci. 69:4696–4702.

Pruneda, I., C. W. Walenciak, B. A. Gardner, H. G. Dolezal, F. N. Owens, and B. Freking. 1999. Pages 165–171 in Time of Implanting Prior to Harvest Effects on Meat Tenderness. Oklahoma Agric. Exp. Stn., Stillwater.

Ranaweera, K. N. P., and D. R. Wise. 1981. The effects of trienbolone acetate implantation of turkeys upon fluid balance and blood chemistry. Br. Poult. Sci. 22:201–208.

Reecy, J. M., J. E. Williams, M. S. Kerley, R. S. MacDonald, W. H. Thornton Jr., and L. M. Wallace. 1994. Abomasal casein infusion enhances the mitogenic activity of serum from protein-restricted steers. J. Nutr. 124:67–77.

Roeder, R. A., S. D. Thorpe, F. M. Byers, G. T. Schelling, and J. M. Gunn. 1986. Influence of anabolic agents on protein synthesis and degradation in muscle cells grown in culture. Growth 50:485–495.

Rooyackers, O. E., and K. S. Nair. 1997. Hormonal regulation of human muscle protein metabolism. Annu. Rev. Nutr. 17:457–485.

Samber, J. A., J. D. Tatum, M. I. Wray, W. T. Nichols, J. B. Morgan, and G. C. Smith. 1996. Implant program effects on performance and carcass quality of steer calves finished for 212 days. J. Anim. Sci. 74:1470–1476.

Sinnett-Smith, P. A., C. A. Palmer, and P. J. Buttery. 1987. Androgen receptors in skeletal muscle cytosol from sheep treated with trenbolone acetate. Horm. Metabol. Res. 19:110–114.

Skjaerlund, D. M., D. R. Mulvaney, R. H. Mars, A. L. Schroeder, M. A. Stachiw, W. G. Bergen, and R. A. Merkel. 1988. Measurment of protein turnover in skeletal muscle strips. J. Anim. Sci. 66:687–698.

Thomson, D. U., R. L. Preston, and K. J. Morrow. 1996. Effect of serum from steers treated with a steroidal implant and somatotropin alone and in combination on in vitro muscle cell proliferation, protein synthesis and degradation. Pages 13–15 in Texas Tech Univ. Tech. Rep. No. T-5-361, Lubbock.

Trenkle, A. 1976. The anabolic effects of estrogens on nitrogen metabolism of growing and finishing cattle and sheep. Pages 79–99 in Anabolic Agents in Animal Production. F. C. Lu and J. Randel, ed. Georg. Thieme Publishers, Stuttgart.

USDA. 1989. Official U.S. Standards for Grades of Carcass Beef. USDA, Washington, DC.

USDA. 1990. Institutional Meat Purchasing Specifications for Fresh Beef. AMS, USDA, Washington, DC.

Vernon, G. G., and P. J. Buttery. 1978. The effect of trenbolone acetate with time on the various responses of protein synthesis of the rat. Br. J. Nutr. 40:563–572.