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Meat Science

Beef color and tenderness response to production systems utilizing additive combinations of growth-promotant technologies

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Abstract

The objective of this study was to compare the influence of beef production systems using additive combinations of growth-promotant technologies on meat quality. Steer calves ($n = 120$) were assigned to 1 of 4 treatments: 1) no technology (NT; control), 2) antibiotic treated (ANT; NT plus therapeutic antibiotics, monensin, and tylosin), 3) implant treated (IMP; ANT plus a series of three implants), and 4) beta-agonist treated (BA; IMP plus ractopamine-HCl). Muscle biopsy samples from the longissimus lumborum were extracted from a subset ($n = 4$ per treatment) of steers to evaluate expression of calpain-1, calpain-2, and calpastatin using real-time RT-PCR. Following carcass chilling, objective color (L^*, a^*, and b^*) was evaluated. The right strip loin was removed from each carcass, portioned into 2.54-cm steaks, and designated to 7, 14, or 21 d postmortem aging periods for analysis of cook loss and Warner–Bratzler shear force (**WBSF**). The anterior face of each strip loin was used for analysis of crude fat and moisture. Treatment influenced ($P < 0.001$) L^*, a^*, and b^*. The NT and IMP treatments had greater ($P < 0.01$) L^* values, ANT was intermediate, and BA had the lowest ($P < 0.01$) L^* values. The NT and IMP treatments had higher ($P < 0.01$) a^* and b^* values compared with ANT, which were higher ($P < 0.01$) than BA. Steaks from implanted steers (IMP and BA) tended ($P \leq 0.067$) to exhibit higher a^* and b^* than steaks from nonimplanted steers. Cattle in the NT and ANT treatments produced steaks with increased ($P < 0.01$) crude fat percentage compared with the IMP and BA treatments, which were similar ($P > 0.05$). Percent moisture of NT steaks was lower ($P < 0.01$) than all other treatments, ANT was intermediate, and IMP and BA were similar ($P > 0.05$) and had the highest ($P < 0.01$) moisture content. Cook loss tended to be greater ($P = 0.088$) for implanted steers (IMP and BA) compared to nonimplanted steers (NT and ANT). Steaks from NT and ANT treatments were more tender ($P < 0.05$) than IMP and BA, which were similar ($P > 0.05$). Thus, WBSF was lower ($P < 0.001$) in nonimplanted than implanted steaks. Expression of calpastatin was increased ($P \leq 0.025$) in ANT and BA treatments, and there was a tendency for expression of calpain-2 to be increased ($P = 0.081$) in ANT compared to NT. These results suggest that production systems with limited use of growth promoting technology produced strip loins with more crude fat, less moisture and cook loss, and improved tenderness.

Keywords: beef, beta agonist, color, growth-promotant technology, implant, tenderness

Introduction

Demand for food production is increasing as the world population continues to grow (AgMRC, 2012). Use of growth-promotant technologies such as feed-grade antimicrobials, antibiotics, implants, and beta-adrenergic agonists could be key to meet this demand through improved animal performance and efficiency (Preston, 1999; Duckett and Andrae, 2001; Jones et al., 2016). Use of ractopamine-HCl (**RH**) has been shown to improve average daily gain and feed efficiency of cattle (Avendaño-Reyes et al., 2006; Walker et al., 2006), while implants increase protein deposition by enhancing both the rate and efficiency of muscle growth (Dayton and White, 2014). Ionophores increase efficiency and performance by modulating the rumen environment (Strydom, 2016). Therefore, these technologies and others could be key to efficiently providing more protein for the growing world demand. However, reports investigating the influence of these technologies on beef quality are mixed (Avendaño-Reyes et al., 2006; Quinn et al., 2008; Strydom et al., 2009). Specifically, the additive effect of varying combinations of growth promoting technologies on beef color and tenderness are unclear.

It is well established that cattle receiving multiple growth implants may experience a reduction in tenderness and overall consumer eating quality (Platter et al., 2003; Lean et al., 2018). Tenderness variability is a critical issue facing the beef industry (Morgan et al., 1991; Koohmaraie and Geesink, 2006), and it is necessary to fully understand the impact of preharvest technologies on this palatability trait. In addition, consumer choice for beef is often based on appearance, and color is a major contributor to acceptability (Altmann et al., 2023). Therefore, the hypothesis was that beef produced with multiple growth-promotant technologies would have less acceptable color, and decreased fat content and tenderness. The objective of this study was to compare

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production systems using additive combinations of growth promoting technologies on beef color, lipid and water content, steak cook loss, tenderness, and expression of genes related to tenderness.

Materials and Methods

Animals

All animal care and experimental protocols were approved by the South Dakota State University (SDSU) Animal Care and Use Committee (approval number 15-091E). A detailed description of the animals, experimental design, and experimental treatments is available in a companion paper by Webb et al. (2020). In brief, Angus × Simmental crossbred steer calves (n = 120) born at the SDSU Antelope Field Station near Buffalo, SD were used. Calves were stratified by birth date, birth weight, and dam age to 1 of 4 treatments: 1) no technology (NT; control); 2) treated with antibiotics and antimicrobials (ANT; NT plus therapeutic antibiotics, fed 300 mg monensin [Rumensin 90, Elanco Animal Health, Greenfield, IN] and 90 mg tylosin [Tylan 40, Elanco Animal Health] per steer daily during the finishing phase); 3) administered implants (IMP; ANT plus a series of three implants including a low-potency suckling calf implant [36 mg zeranol; Ralgro, Merck Animal Health, Madison, NJ] at an average of 74 ± 12 d of age, a moderate-potency initial feedyard implant [80 mg trenbolone acetate and 16 mg estradiol; Revalor-IS, Merck Animal Health] at an average of 235 d of age, and a high potency finishing implant [200 mg trenbolone acetate and 20 mg estradiol; Revalor-200, Merck Animal Health] at an average of 330 d of age); and 4) administered a beta-agonist (BA; IMP plus fed 200 mg RH [Optaflexx 45; Elanco Animal Health] per steer daily for the last 30 d before harvest).

Carcass Evaluation and Sample Collection

Upon completion of all live animal procedures (see Webb et al., 2020 for details), steers were tracked individually through harvest at a commercial processing facility. Following carcass chilling (approximately 24 h as per plant protocols), carcasses were ribbed between the 12th and 13th rib, and the exposed longissimus dorsi (**LD**) was allowed to bloom for approximately 30 min before objective color (L^*, a^*, and b^*) measurements were recorded. A Minolta colorimeter (model CR-310; Minolta Corp., Ramsey, MJ; 50 mm diameter measuring space and D65 illuminant) was used to obtain measurements from two locations of the exposed LD (medial and lateral) and averaged for each carcass. The right strip loin (IMPS 180) was collected from all carcasses (n = 120) and transported under refrigeration (2.2 °C) to the SDSU Meat Laboratory in Brookings, SD. Strip loins were trimmed to 0.64 cm of external fat, the connective tissue, gluteus medius, and multifidus dorsi were removed so that only the longissimus lumborum (**LL**) remained. The most anterior portion of the LL was faced to obtain a square anterior edge and the remaining portion was fabricated into 2.54-cm steaks. The anterior face of the LL was aged for 14 d postmortem and used to evaluate crude fat percentage. The three most anterior steaks from each strip loin were assigned to 7-, 14-, or 21-d postmortem aging periods and vacuum-sealed for analysis of percent cook loss and Warner–Bratzler shear force (WBSF). Vacuum-sealed samples were aged in the absence of light at 2 to 3 °C and immediately after each specified aging period was attained were frozen (–20 °C) and checked regularly for seal integrity until thawed for evaluation of percent moisture, crude fat, percent cook loss, and tenderness.

Moisture and Crude Fat Percentage

Crude fat percentage was determined using ether extraction methods described by Webb et al. (2017). Briefly, steaks were thawed slightly and prepared for powdering using a Waring commercial blender (model 51BL32; Waring Laboratory Division, Lancaster, PA). Once powdered, individual samples were stored in bags (Whirlpack; Nasco, Fort Atkinson, WI) and frozen (–20 °C). For analysis, duplicate powdered samples (5 g) were weighed into tins, covered with filter papers, and dried in an oven at 101 °C for 24 h. Once dried, samples were placed into desiccators for 1 h prior to recording the nonextracted weight for calculation of percent moisture. Samples were extracted according to the AOAC International (Horwitz, 2000; method 960.39) with the exception that the Soxhlet extractor (model 80068-154; Chemglass Life Sciences LLC, Vineland, NJ) was used with petroleum ether instead of a Goldfisch apparatus. Ether extraction was conducted for 60 h followed by evaporating samples at room temperature before placing the tins into the oven for 4 h at 101 °C (Bruns et al., 2004). Dried, extracted samples were put into desiccators for 1 h prior to re-weighing. Crude fat was calculated by determining the difference among the pre- and postextraction sample weight and was expressed as a percent of the pre-extracted sample weight.

Percent Cook Loss and Warner–Bratzler Shear Force

Steaks designated for WBSF determination were thawed for 24 h at 4 °C. Prior to cooking, each raw steak was weighed, placed on an electric clam shell grill (George Forman 9 Serving Classic Plate Grill, Model GR2144P, Middleton, WI), and cooked to a target internal peak temperature of 71°C. During cooking the MicroNeedle probe of an AquaTuff thermometer (Model 35140, Cooper-Atkins Corporation, Middlefield, CT) was placed into the geometric center of each steak to continuously monitor the temperature and peak temperature was recorded. After cooking, each steak was cooled for 24 h at 4 °C before removing six cores (1.27 cm in diameter) parallel to the muscle fiber orientation (AMSA, 2015). A single, peak shear force measurement was obtained for each core using a Warner–Bratzler machine (G-R Electric Manufacturing Company, Manhattan, KS). The peak shear force was recorded for each core and averaged to obtain a single shear force value per steak.

Gene Expression

Biopsy samples (~40 mg) were collected from the LL of a subsample (n = 4 per treatment) of steers for analysis of gene expression. Biopsies were collected from NA and IMPL 6 d before harvest and NHTC and IMBA 5 d before harvest. An area of approximately 12.7 cm^2 was shaved using surgical precision blades and scrubbed with a povidone–iodine solution, followed by a 70% alcohol solution. A total of 5 mL of lidocaine was injected in a circle of beads around the planned incision site. After local anesthesia was established, a 10 mm incision was made using a sterile No. 11 scalpel, and a BARD Magnum Reusable Core Biopsy System with a 12 G × 10 cm needle was used to collect muscle tissue (C.R. Bard, Inc., Tempe, AZ). The needle was inserted into the incision site to collect tissue samples and repeated (5 to 7 times

per steer). Tissue was immediately removed from the biopsy needle and snap frozen in liquid N before storage at –80 °C. The injection site was sprayed with Vetericyn antimicrobial topical spray (Vetericyn, Rialto, CA) and steers were closely monitored until fully recovered. No ill effects were observed in any steers on either biopsy date.

RNA Extraction, cDNA Conversion, and Real-Time RT-PCR

Snap frozen samples were powdered in liquid N using a mortar and pestle and approximately 60 mg of sample were placed into 1.5 mL tubes containing 700µL of QIAzol 154 Lysis Reagent. Total RNA was extracted from samples using the miRNeasy Mini Kit (Catalog No. 217004 QIAGEN, Germany). Following the miRNeasy Mini Kit quick-start protocol, RNA was separated from genomic DNA. The concentration and purity of RNA was evaluated spectrophotometrically (Nanodrop 2000, Thermo Scientific, Wilmington, DE) and RNA concentration was diluted to 200 ng/µL. A high-capacity cDNA Reverse Transcription Kit (Part #4368814, Applied Biosystems, Foster City, CA) was used to convert RNA to cDNA using a thermal cycler (MyCycler Thermocycler #170-9703, BioRad Laboratories, Hercules, CA) at the parameters recommended by the manufacturer set at 1 cycle at 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 min. The cDNA was diluted to 50 ng/µL using RNAase-free water, real-time PCR was performed to evaluate the expression of three genes associated with the calpain system (calpain-1, calpain-2, and calpastatin). The National Center for Biotechnology Information (NCBI; United States Library of Medicine, Bethesda, MD) database was utilized to identify messenger RNA sequences. GeneBank accession numbers were then used to design primers using PrimerQuest software (Integrated DNA Technologies, Coralville, IA). Accession numbers, forward primer sequences, and reverse primer sequences for each housekeeping gene (EEF1A2 and SF3A1) are presented in Table 1. Real-time PCR was carried out using RT2 Real-Time SYBR Green/ROX PCR Master Mix (PA-012-24, SABiosciences, Frederick, MD) with appropriate forward and reverse primers (10 nM), and 1 µL diluted cDNA. Assays were performed using a Mx3005P thermal cycler (Agilent Technologies, Stratagene Product Division, Waldbronn, Germany) with parameters recommended by the manufacturer: 95 °C for 10 min and then 40 cycles of 95 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s. Reaction specificity was determined by melting curves for each amplicon after completion of amplification.

Statistical Analysis

Treatments were evaluated using PROC MIXED of SAS (version 9.4, SAS Inc., Cary, NC). Meat color (L^*, a^*, and b^*), percent moisture and crude fat were evaluated in a completely randomized design with treatment as the fixed effect, carcass as the experimental unit, and damage included as a covariate. For WBSF and cook loss, effects of treatment and aging day were considered fixed effects in a factorial treatment structure. Aging day was considered a repeated measure and the variance–covariance matrix was chosen using the Schwarz's Bayesian Information Criterion goodness of fit statistic. Dam age and peak cooking temperature were included as covariates for cook loss and WBSF. In all cases, the denominator degrees of freedom were approximated using the Kenward–Roger option in the model statement. Least square means and SEM were computed for all variables and separated using least significant differences (PDIFF) when tests for fixed effects were significant at $P \leq 0.05$. For all statistical models, a preplanned contrast of nonimplanted (NT + ANT) vs. implanted (IMP + BA) treatments was tested.

Fold change differences in gene expression between NT, which served as the control, and ANT, IMP, or BA were analyzed using the Relative Expression Software Tool (REST; 2008, Corbett Research & M. Pfaffl, Technical University Munich) according to the procedures of Pfaffl (2001). Relative expression is dependent upon the expression ratio of a target gene compared with a reference gene and is accepted for most investigations of physiological change in the level of gene expression (Mohrhauser et al., 2015). Target gene expression was standardized by the average of two nonregulated reference genes. The expression ratio was tested for statistical significance using a Pairwise Fixed Reallocation Randomization Test (Pfaffl et al., 2002). In this study, EEF1A2 and SF3A1 were used as reference genes for each LL muscle biopsy sample (Table 1). Responses were considered significant at $P \leq 0.05$, and tendencies were considered at $P > 0.05$ to $P \leq 0.10$.

Table 1. Primer sequences for housekeeping genes and genes of interest

Gene		Primer sequence	Accession number
EEF1A2[1,2]	Forward	5′-GGTACTGGACAAGCTGAAGG-3′	NM_001037464
	Reverse	5′-GCGTCGATGATGGTGATGTA-3′	
SF3A[1,3]	Forward	5′-GCCCGTGGTGGGTATTATTTA-3′	NM_001081510
	Reverse	5′-TGTTGATCTCGTTCTGTCGTATC-3′	
Calpastatin	Forward	5′-GCCAAAGGAACACACAGAGCCAAA-3′	NM_001030318
	Reverse	5′-TTCTCTGATGGTGGCTGCTCACTT-3′	
Calpain-1	Forward	5′-ATTTCCAGCTGTGGCAGTTTGGTG-3′	NM_174259
	Reverse	5′-TCACCTTGGCATAGGCTTTCTCCA-3′	
Calpain-2	Forward	5′-TGACCCAAACTGGGCATCTGTCTA-3′	NM_001103086
	Reverse	5′-AAACAAGCTTGGGTGGTTTCCCTG-3′	

[1]Housekeeping gene.
[2]EEF1A2, eukaryotic translation elongation factor 1.
[3]SF3A1, splicing factor 3.

Results and Discussion

Production system treatments influenced L^* values ($P < 0.001$), but the implant administration contrast was not significant ($P > 0.05$). The LD from NT and IMP displayed similar ($P > 0.05$) L^* values and were lightest in color, L^* of ANT was intermediate ($P < 0.05$), and L^* of BA was darkest ($P < 0.05$) in color (Table 2). In contrast, Garmyn et al. (2014) reported L^* values were similar between muscles from steers fed RH and a nonsupplemented control. Moreover, Avendaño-Reyes et al. (2006) observed lighter steaks from carcasses of cattle supplemented with RH compared to a control.

Treatment also influenced a^* values ($P < 0.001$). The LD from NT and IMP displayed similar a^* values ($P > 0.05$) and were redder in color than ANT and BA ($P < 0.05$). The a^* values of ANT were intermediate ($P < 0.05$), and a^* of BA was least red ($P < 0.05$) in color (Table 2). Additionally, a^* of the LD from implanted steers (IMP and BA) tended ($P = 0.067$) to be less red than nonimplanted steers (NT and ANT). Garmyn et al. (2014) also reported that a^* values were decreased due to RH supplementation of steers in comparison to a nonsupplemented control. Additionally, Reiling and Johnson (2003) conducted a retail display study and determined steaks from implanted cattle had reduced a^* values (at day 0) compared to steaks from the nonimplanted control.

The LD from NT and IMP were similar ($P > 0.05$) but had increased ($P < 0.05$) b^* values, or were more yellow in color than ANT, which was intermediate and greater ($P < 0.05$) than BA. Further, b^* of the LD from implanted steers (IMP and BA) tended ($P = 0.067$) to be less yellow than nonimplanted steers (NT and ANT). At day 0 of retail display, Reiling and Johnson (2003) determined that steaks from steers implanted with zeranol and re-implanted with a combination implant (trenbolone acetate and estradiol) had lower b^* values compared to a nonimplanted control, which is in agreement with the current orthogonal contrast result. However, Reiling and Johnson (2003) reported that steers implanted and re-implanted with the same combination implant were similar to the control, which is consistent with the comparison of NT and IMP in the current study. Hilton et al. (2009) evaluated carcass color from cattle that had monensin and tylosin removed during the finishing phase and determined b^* values were not influenced. In contrast to the current results for BA, Avendaño-Reyes et al. (2006) reported no difference in RH supplementation on b^* values in comparison to a nonsupplemented control. Moreover, Woerner et al. (2011) determined initial implanting, terminal implanting, and RH supplementation did not influence color (L^*, a^*, b^*) values. However, these studies (Avendaño-Reyes et al., 2006; Woerner et al., 2011) had variations in breed type (Charolais and Brangus), animal age (calf-fed), implant protocol (progesterone and estradiol benzoate), and timing of administration in comparison to the current study.

Treatment influenced ($P < 0.001$) crude fat percentage (Table 2). The NT and ANT treatments were similar ($P > 0.05$) but greater ($P < 0.05$) than IMP and BA, which were not different ($P > 0.05$). Moreover, the implant vs. nonimplant contrast indicated reduced ($P < 0.001$) fat content in steaks from implanted steers compared to nonimplanted steers. This is supported by greater marbling score in the nonimplanted treatments from these steers reported by Webb et al. (2020). Steers from the NT and ANT treatments also produced lighter hot carcass weights than the IMP and BA (Webb et al., 2020). Treatment also influenced ($P < 0.001$) moisture percentage. Steaks from NT steers had the lowest ($P < 0.05$) moisture, while IMP and BA steaks were similar ($P > 0.05$) and had the greatest moisture content ($P < 0.05$, Table 2). Moisture percentage of steaks from ANT steers were intermediate and different ($P < 0.05$) from all other treatments. In addition, the implant vs. nonimplant contrast indicated greater ($P < 0.001$) moisture content in steaks from implanted steers compared to nonimplanted steers. These results suggest that use of monensin and tylosin and successive implantation additively shifted percentages of crude fat and moisture in comparison with the NT control that received no growth promoting technologies. The lack of further change with the addition

Table 2. Main effect least square means for effect of production system on meat color, percent fat, percent moisture, cook loss, and tenderness

Variable	Treatment[1]				SEM	*P*-value[2]	*P*-value[3]
	NT	ANT	IMP	BA			
L^* [4]	43.90[c]	42.70[b]	43.84[c]	41.81[a]	0.327	<0.001	0.137
a^* [5]	26.37[c]	24.73[b]	26.38[c]	24.09[a]	0.178	<0.001	0.067
b^* [6]	11.87[c]	10.57[b]	11.95[c]	10.03[a]	0.129	<0.001	0.060
Crude fat, % [7]	7.38[b]	7.11[b]	5.49[a]	5.89[a]	0.307	<0.001	<0.001
Moisture, % [7]	69.67[a]	70.39[b]	71.23[c]	71.20[c]	0.231	<0.001	<0.001
Cook loss, % [8]	18.97	18.59	19.93	19.37	0.527	0.269	0.088
WBSF, kg [9]	2.01[a]	1.94[a]	2.49[b]	2.63[b]	0.078	<0.001	<0.001

[1]NT = received no technology, ANT = administered antibiotics but no other technology, IMP = administered antibiotics and implants, BA = administered antibiotics, implants and a beta-agonist.
[2]Probability of a greater *F* for test of treatment fixed effect.
[3]Probability of a greater *F* for contrast of nonimplanted (NT + ANT) vs. implanted (IMP + BA) treatments.
[4]0 = black and 100 = white.
[5]Negative values = green and positive values = red.
[6]Negative values = blue and positive values = yellow.
[7]Percentage of raw steak composition.
[8]Percent weight loss after cooking; peak internal temperature included as a covariate.
[9]Kilogram of force measured by Warner–Bratzler shear force; peak internal temperature included as a covariate.
[a,b,c]Least squares means within row with different superscripts differ ($P \leq 0.05$) for treatment fixed effect.

of RH to IMP indicates this technology did not influence fat and moisture content beyond the effects of the other growth promoting technologies. The decrease in crude fat percentage because of IMP and BA compared to NT was expected, as was the inverse effect on moisture percentage. Although utilizing nonpregnant cull cows, Cranwell et al. (1996) agreed that use of an implant (200 mg trenbolone acetate) decreased crude fat percentage and increased moisture percentage. In contrast to the current BA treatment responses, Schroeder et al. (2005) evaluated steaks from heifers supplemented with RH (10, 20, or 30 ppm) in comparison to a nonsupplemented control and determined there was no influence on crude fat or moisture percentages.

Growth-promotant technology treatments and aging period did not interact ($P > 0.05$) for cook loss or WBSF. Technology treatments did not influence ($P = 0.269$) percent cook loss (Table 2). There was a tendency for cook loss in steaks from implanted steers to be greater than nonimplanted steers ($P = 0.088$). Tenderness of all steaks improved ($P < 0.001$) between days 7 and 14 of aging (2.45 ± 0.043 kg vs. 2.20 ± 0.043 kg at 7 and 14 d, respectively (lsmean ± SEM]), but no further improvement ($P > 0.05$) was observed on d 21 (2.15 ± 0.043 kg, lsmean ± SEM). Steaks from NT and ANT were similar ($P > 0.05$) and more tender ($P < 0.05$) than IMP and BA, which were similar ($P > 0.05$). This effect was corroborated by the implant vs. nonimplant contrast ($P < 0.001$). However, it should be noted that steaks from all treatments could be certified very tender (≤3.9 kg) as a marketing claim according to ASTM (2011). The extent of the impact of implants on tenderness varies. A decrease in tenderness of steaks from implanted cattle has been reported widely in the literature (Morgan, 1997; Roeber et al., 2000; Platter et al., 2003). Other studies have reported minimal negative influences on steak tenderness from cattle administered successive implants (Gerken et al., 1995; Nichols et al., 2002). In the present study, the differences in shear force values of the strip loin could be considered negligible since all treatments would likely be considered tender by consumers (Miller et al., 2001; Destefanis et. al., 2008), however, the impact of treatments on other muscles in the carcass is unknown.

While some studies have indicated that RH supplementation negatively influenced beef tenderness (Avendaño-Reyes et al, 2006 [LD evaluated]; Gruber et al., 2008 [LL evaluated] Strydom et al., 2009 [semitendinosus evaluated]; Scramlin et al., 2010 [LL evaluated]; Boler et al., 2012 [LL evaluated]; Arp et al., 2013 [LL evaluated]), a few reports (Platter et al., 2008 [LL evaluated]; Arp et al., 2013 [LL evaluated]) suggest that a low dose of RH (200 mg RH·steer^{-1}· d^{-1}; similar to the current study) did not decrease steak shear force in comparison with a non-RH control. Moreover, trained sensory panels have detected an increase in connective tissue in steaks from carcasses of steers supplemented with RH (400 mg RH·steer^{-1}· d^{-1}) and reported those steaks to be tougher in comparison to a control (Arp et al., 2013). Perhaps implantation masked the shear force response from a low dose of RH reported by Arp et al. (2013) and in the current study (IMP vs. BA). Woerner et al. (2011) evaluated sequential additive combinations of initial and terminal implants plus 200 mg RH·hd^{-1}· d^{-1} for calf-fed steers and heifers. In contrast with the current results, WBSF values were not influenced by the initial or terminal implants; however, RH supplementation increased mean WBSF value by 0.23 kg. It should be noted that the WBSF values from Woerner et al. (2011) were reported to be at least a full kg higher than the steaks from the cattle in any treatment of the current study.

Expression of calpain-1 was not influenced ($P = 0.84$) by ANT, while calpastatin was upregulated ($P < 0.05$) and calpain-2 tended to be upregulated ($P < 0.10$) in samples from the ANT treatment compared to NT (Table 3). Limited research exists evaluating the effect of monensin and tylosin on expression of the calpain system in muscle. Hilton et al. (2009) evaluated the withdrawal of monensin and tylosin while feeding zilpaterol hydrochloride. During the last 35 d prior to harvest, expression of calpain or calpastatin was not influenced by removal of these products from the diet. However, the current study did not evaluate change in expression when these technologies were removed from the diet, which could explain the differing results.

There was no difference ($P > 0.10$) in calpain-1, 2, or calpastatin expression between the IMP and NT treatments (Table 3). Gerken et al. (1995) also determined implanting with either an estrogenic, an androgenic, or a combination implant did not influence gene activity of calpain-1 or -2 in comparison to a nonimplanted control. However, steers receiving either a single estrogenic or a combination implant had increased calpastatin activity compared to a nonimplanted control (Gerken et al., 1995). Differences in calpastatin response of Gerken et al. (1995) and the present study may be related to differences in specific implants administered or the difference between assays utilized to quantify differences in calpastatin (activity vs. gene expression). Additional differences, such as time of sampling can add variation to the reported data. Reichhardt et al. (2022) reported increased calpastatin protein expression as early as two days post

Table 3. Relative expression of genes in the longissimus lumborum muscle of steers raised with additive combinations of growth-promotant technologies

Gene	Treatment[1]								
	ANT			IMP			BA		
	Fold Change[2]	95% CI	*P*-value	Fold Change	95% CI	*P*-value	Fold Change	95% CI	*P*-value
Calpain-1	0.886	0.177–5.110	0.840	1.266	0.568–2.823	0.519	1.595	0.789–2.574	0.110
Calpain-2	1.601	0.932–3.997	0.081	1.020	0.718–1.447	0.784	1.120	0.365–3.520	0.733
Calpastatin	1.560	1.095–2.266	0.010	1.042	0.853–1.187	0.631	1.615	1.318–2.029	0.025

[1]NT = received no technology, ANT = administered antibiotics but no other technology, IMP = administered antibiotics and implants, BA = administered antibiotics, implants and a beta-agonist.
[2]Fold change compares steers within production system to steers receiving no technology (NT), fold change greater than 1 denotes increased expression within production system.

implantation. Samples in the current study were collected 5 or 6 d before harvest. Gene expression data 5 or 6 d before harvest should be more indicative of the biochemical changes observed during the conversion of muscle to meat.

Expression of calpain-1 and -2 was not influenced ($P > 0.10$) by BA, while expression of calpastatin was upregulated ($P = 0.025$) in the BA treatment compared with NT (Table 3). Others have also reported beta-adrenergic agonist (**β-AA**) supplementation up-regulated calpastatin expression and confirmed that calpastatin expression increased with β-AA induced muscle hypertrophy (Killefer and Koohmaraie, 1994). Walker et al. (2010) extracted muscle biopsies from the biceps femoris and longissimus muscle of 16 steers administered an implant (120 mg trenbolone acetate and 24 mg estradiol-17β) and fed 200 mg RH·steer^{-1}·d^{-1} for 29 d and did not observe any difference in expression of calpastatin when compared to steers only implanted and not fed RH. It is not surprising that use of β-AA in this study increased gene expression of calpastatin as it has repeatedly been documented to increase calpastatin activity and potentially cause new collagen cross-links, which may decrease meat tenderness (Goll et al., 1997; Strydom et al., 2009; and Roy et al., 2015).

Implications

Cattle "raised without antibiotics" (NT treatment), or "raised without the use of hormones" (NT and ANT) produced strip steaks that contained more crude fat and were more tender in comparison to steaks produced from cattle that also received growth promoting implants (IMP treatment) and RH (BA treatment). However, steaks from all treatments in this study were considered acceptable for tenderness indicating all production systems can meet consumer acceptability. Thus, performance benefits from growth promoting implants and RH were not realized at a meaningful detriment to meat tenderness of the strip loin compared to nonimplanted cattle. Based on these results, producers may expect to capture heavier carcass weights without sacrificing meat quality, leading to improved overall economic value, however additional work to characterize the influence of these treatments on other muscles in the carcass is warranted. Consumer awareness and interest in production practices is growing (e.g., desire to avoid exogenous hormones in meat), thus further research is needed to understand consumer acceptance and demand for beef raised with these growth-promotant technologies.

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