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J Anim Sci 2007. 85:430-440.
doi: 10.2527/jas.2006-280

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The effect of anabolic implants on intramuscular lipid deposition in finished beef cattle

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ABSTRACT: Two experiments were conducted to determine the effects of anabolic implants on performance, changes in ultrasound measurements, carcass quality, cellularity of i.m. and s.c. adipose depots, and mRNA expression of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), and lipoprotein lipase (LPL) in i.m. adipose tissue of finished beef cattle. Angus heifers (experiment 1: n = 10; 411 kg of BW) and steers (experiment 2: n = 18; 279 kg of BW) were randomly allotted as control (C) or implanted with Synovex-Plus (SP) at d 0 and midway through the finishing period. The cattle were fed a high-concentrate diet and were weighed at approximately 28-d intervals. Heifers and steers were finished for 108 and 133 d, respectively. At slaughter, a section of the LM (sixth to ninth rib) was removed, and i.m. adipose tissue was dissected for mRNA analysis. Subcutaneous and i.m. adipose tissues also were collected for determination of cellularity. At 48 h postmortem, carcass data were collected, and a steak (12th rib) was removed for analysis of lipid and fatty acid composition. Body weight did not differ ($P > 0.10$) between treatments until after reimplanting of the heifers (d 55) or steers (d 73). Average daily gain was 36 and 16% faster ($P \leq 0.01$) for implanted heifers

and steers, respectively, compared with their control counterparts. Implanting resulted in larger ($P \leq 0.10$) HCW and LM area for heifers and steers. However, implanting did not affect ($P > 0.10$) dressing percent, fat thickness, percentage of KPH, yield grade, or marbling score. Intramuscular lipid content and concentrations of major fatty acids did not differ ($P > 0.10$) between treatments. Percentage of SC adipocytes was greater at larger diameters ($>150 \mu\text{m}$), whereas the majority of i.m. adipocytes were at small to middle diameters (50 to 150 μm). The number of i.m. adipocytes per gram of tissue was greater ($P < 0.05$) for SP than C and also were greater ($P < 0.05$) than the number of s.c. adipocytes in SP heifers. In experiment 2, adipocytes per gram of tissue tended to be greater ($P = 0.07$) for SP than C and were greater ($P < 0.01$) for i.m. than s.c. In experiment 1, average cell diameter and volume did not differ ($P > 0.10$) between treatments and tissues, but in experiment 2 both cellularity traits were greater ($P < 0.01$) for s.c. than for i.m.. Implanting did not alter mRNA expression of ACC, SCD, or LPL in i.m. adipose tissue. This study shows that anabolic implants do not appear to have direct effects on i.m. lipid deposition.

Key words: adipose cellularity, beef, implant, lipogenic enzyme, marbling

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J. Anim. Sci. 2007. 85:430–440
doi:10.2527/jas.2006-280

INTRODUCTION

Anabolic implants are integrated into the management practices of the finishing phase of US beef production to enhance animal performance (Samber et al., 1996; Duckett and Andrae, 2001) and carcass muscle yield (Johnson et al., 1996a,b; Roeber et al., 2000). Return on investment from the use of implants is generally

positive, but its effect on carcass quality and palatability has not been consistent across studies.

Some trials have shown that implants have no effect on quality grade (Gerken et al., 1995; Johnson et al., 1996a), but a lower quality grade as a consequence of reduced marbling score or advanced skeletal maturity, or both, has been reported by others (Belk, 1992; Duckett et al., 1997). Specifically, a reduction in marbling score has been observed in some trials (Morgan, 1997; Roeber et al., 2000) in which the cattle were implanted with a combination of trenbolone acetate and estradiol. A related study showed that a combination implant reduced total fatty acid content and altered fatty acid composition although differences were not significant when expressed on a gravimetric basis (Duckett et al., 1999).

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Received May 1, 2006.

Accepted September 1, 2006.

A summary of implant usage by Duckett and Andrae (2001) showed an inverse relationship ($r^2 = 0.68$) between marbling score and LM area. As LM area increased, marbling score decreased, suggesting that implants have an indirect effect on i.m. lipid deposition (i.e., dilution of i.m. fat in a larger LM area). Moreover, i.m. fat content of LM has been shown to be decreased by early administration of an estradiol-trenbolone acetate implant (Bruns et al., 2005). However, assessment of the effects of anabolic implants on i.m. adipose cellularity and mRNA expression of enzymes related to lipid accretion and deposition is lacking.

Therefore, the objective of this study was to determine the effect of anabolic implants on changes in ultrasound measurements (LM area, fat thickness, and i.m. fat percentage), carcass yield and quality, cellularity of i.m. and s.c. fat depots, and i.m. adipose tissue mRNA expression of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), and lipoprotein lipase (LPL).

MATERIALS AND METHODS

Animals, Diets, Ultrasound Measures, and Experimental Procedures

Two experiments were conducted to determine the effects of anabolic implants on lipid deposition in finished cattle. These experiments were approved by the University of Georgia Animal Care and Use Committee. Angus heifers ($n = 10$; 411 kg of BW; 19 mo of age) were used in the first experiment, and steers ($n = 18$; 279 kg of BW; 10 mo of age) of the same genotype were finished in the second experiment. The heifers and steers were purchased from a herd sired by bulls with a high-marbling EPD (marbling EPD $\geq +0.3$). Neither the heifers nor the steers had been implanted before purchase. After purchase, the cattle were transported to the University of Georgia (UGA) Wilkins Beef Cattle Unit.

The same protocols were followed in both experiments except for the addition of Warner Bratzler shear force (WBSF) determination for LM in experiment 2. After an adjustment period, cattle were randomly allotted to serve as control (C) or implanted with Synovex-Plus (SP; 28 mg estradiol benzoate and 200 mg of trenbolone acetate; Fort Dodge Animal Health, Overland Park, KS). In the heifer experiment, the SP group was implanted on d 0 and 55. In the second experiment, SP steers were implanted on d 0 and 73. Cattle in both experiments were fed a corn-cottonseed meal diet (12.5% crude protein, 1.2 Mcal of NE_g/kg , as-fed). In the first experiment, heifers were weighed on d 0, 27, 55, 80, and 108, and in the second experiment, steers were weighed on d 0, 28, 57, 73, 105, and 133. Real-time ultrasound measurements of LM area (ULMA), fat thickness (UFT), and i.m. fat (UIMF) percentage at each weighing time were gathered, except on d 73 in Exp. 2.

An Association of Ultrasound Practitioners-certified technician collected images using an Aloka 500V ma-

chine with an Aloka UST-5049-3.5 (17.2-cm linear) probe (Corometrics Medical Systems, Wallingford, CT). Images were interpreted using Beef Information Manager software (version 3.0; Critical Vision Inc., Atlanta, GA).

Slaughter, Carcass Traits, and Sample Collection

At the completion of each finishing experiment (approximately 50 to 60 d after reimplantation), cattle were transported to the UGA Meat Science and Technology Center, where they were humanely slaughtered under federal inspection. Slaughter weight was recorded immediately before slaughter to be used in calculating the dressing percent. At <15 min after exsanguination, a portion of the LM (sixth to ninth rib) was removed, and i.m. adipose tissue was dissected, frozen in liquid nitrogen, and stored at -70°C for subsequent mRNA analysis. Samples of i.m. and s.c. (sixth to ninth rib) adipose tissues were also collected for tissue cellularity measurements.

Carcasses were chilled at 4°C for 48 h, and trained UGA personnel recorded HCW, actual and adjusted fat thickness, LM area, percentage KPH, marbling score, and skeletal and lean maturity. Yield and quality grades were calculated according to USDA standards (USDA, 1997). A steak (2.54-cm thick) was removed from the LM between the 11th and 12th rib of the right side of each carcass, trimmed of all external fat, pulverized in liquid nitrogen, and stored at -20°C for lipid analysis. In Exp. 2, boneless strip loins (IMPS #180) were fabricated from 1 side of each carcass. All external fat was removed from the strip loin before slicing into 2.54-cm thick steaks. Steaks were then vacuum-packaged and assigned to 1 of 5 aging times (1, 3, 7, 14, or 21 d). After aging, steaks were frozen at -20°C until WBSF determination.

Muscle Lipid Analysis

Total lipids were extracted according to a modified method of Folch et al. (1957). Before this study, it was determined that a 1:10 ratio of sample to chloroform:methanol (2:1, vol/vol) was sufficient for total lipid extraction (Smith, unpublished data). Lipids were transmethylated following the procedure of Park and Goins (1994). Fatty acid composition was determined by using a gas chromatograph; Agilent 6850, Agilent Technologies, Wilmington, DE) equipped with a 100-m Supelco SP2560 capillary column (0.25-mm i.d. and 0.20- μm film thickness, Supelco, Bellefonte, PA) and following the gas chromatograph conditions detailed by Duckett et al. (2002). Methyl tricosanoate (C23:0) was included as an internal standard to quantify fatty acids.

Warner-Bratzler Shear Force Determination

Warner-Bratzler shear force of strip loin steaks was evaluated according to the AMSA (1995) guidelines. Steaks were thawed for 24 h at 4°C , cooked to an inter-

nal temperature of 40°C, turned, and cooked to a final internal temperature of 71°C on a Farberware Open Hearth Broiler (Farberware Inc., Bronx, NY). Steaks were then placed on plastic trays and allowed to equilibrate to room temperature (~22°C). Six cores (1.27 cm in diam.) were removed from each steak, parallel to the muscle fiber orientation. Each core was sheared once through the center, perpendicular to the muscle fiber orientation, with a Warner-Bratzler shear machine (G-R Elec. Mfg., Manhattan, KS). The 6 shear force values from each steak were averaged for statistical analysis.

Adipose Tissue Cellularity

Cellularity of i.m. and s.c. adipose tissues was determined according to the procedures outlined by Mersmann and MacNeil (1986). Duplicate samples (50 mg) of i.m. and s.c. adipose tissue were fixed in osmium tetroxide and dissociated in urea. The number of adipocytes, which ranged from 20 to 240 μm in size, were counted by a Coulter Counter (Coulter Electronics, Hialeah, FL). However, only counts of adipocytes having a diameter of >30 μm were included in the calculation for cell number, diameter, and volume (Lee et al., 1994).

Ribonuclease Protection Assay (RPA) of ACC, SCD, and LPL mRNA from i.m. Adipose Tissue

Total RNA was isolated from i.m. adipose tissue with the QIAzol lysis reagent (RNeasy Lipid Tissue Midi Kit, Qiagen Inc., Valencia, CA) following the nonisotopic RPA procedures outlined by Lee et al. (2002). Antisense biotin-labeled riboprobes were synthesized by reverse-transcription PCR. In a 25- μL volume, 2 μg of total RNA was reverse transcribed with 200 U of reverse transcription (Promega, Madison, WI) at 37°C for 1 h. The cDNA synthesized was amplified using primer pairs for which the antisense primers contained the bacteriophage T7 promoter sequence (27 bp) at the 5' end (Kain et al., 1991). The primer sequences for ACC, SCD, and LPL were reported by Lee et al. (2002) and Bonnet et al. (2001). The primer pairs were synthesized at the Molecular Genetics Instrumentation Facilities (UGA, Athens, GA). The sequences of the sense and antisense primers used for ACC, SCD, LPL, and β -actin are shown in Table 1.

The PCR reactions were completed in a 50- μL volume containing 5 μL of the reverse transcription product, 2.5 mM MgCl_2 , 200 μM dNTPs (Sigma Chemical Co., St. Louis, MO), 400 nM each of sense and anti-sense primers, and 2.5 U of Taq DNA polymerase (Promega). The thermal settings were as follows: 1 cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 60 s, 58°C for 60 s, and 72°C for 60 s, with a final extension at 72°C for 5 min. An in vitro transcription was accomplished using a MAXIscript labeling kit (Ambion Inc., Austin, TX). The riboprobe reaction, in a 20 μL total volume, contained 5 μL of PCR products, 1 \times buffer, T7 RNA polymerase, 0.5 mM each of ATP, CTP, and GTP

with 0.3 mM of UTP, and 0.2 mM biotin-labeled-16-UTP (Roche, Mannheim, Germany). The reaction was conducted for 1 h at 37°C then incubated with DNase I for 15 min at 37°C. Transcripts were denatured (95°C for 3 min) and separated on 6% acrylamide-7 M urea, denaturing polyacrylamide gels (Invitrogen, Carlsbad, CA). After staining with ethidium bromide, the full-length riboprobe was excised and eluted from the gel in probe elution buffer (Ambion) overnight at 37°C, precipitated, and quantified at 260/280 nm.

The RPA reaction was carried out using a RPA III kit (Ambion Inc.). One multiprobe (ACC, SCD, LPL, and β -actin standard) RPA reaction per animal was completed, as the expected product sizes, based on the probe sequence, were at least 10% different in bp length between probes. Total RNA and 10-fold molar excess riboprobe was denatured (95°C for 3 min) and then allowed to hybridize at 56°C overnight in 10 μL of hybridization buffer. The reactions were digested with RNase and then, through a simultaneous Ambion-patented procedure, RNase was inactivated, and protected RNA was precipitated.

The protected RNA was separated by electrophoresis through a 6% acrylamide-7 M urea, denaturing polyacrylamide gel (Invitrogen) and electrophoretically transferred (XCell SureLock Mini-Cell with XCell II Blot Module Kit, Invitrogen) onto a positively charged nylon membrane (BrightStar-Plus, Ambion) with 0.5 \times TBE at 400 mA for 1 h. Once the membrane was UV-crosslinked, nonisotopic detection was performed using a BrightStar BioDetect kit (Ambion), in which the membrane was incubated with streptavidin-alkaline phosphatase for 30 min and CDP-Star for 5 min at room temperature. The chemiluminescence of the membrane was evaluated using an Alpha Innotech Imager, and the image was analyzed with Fluorchem 8000 software (Alpha Innotech, San Leandro, CA). The intensity of the target band was expressed as a percentage of the β -actin standard band intensity on each gel lane to calculate a ratio that was then statistically compared for treatment effects.

Statistical Analysis

Data were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC), with individual animal serving as the experimental unit. Body weight, ADG, and ultrasound measurements were analyzed by 1-way ANOVA for each time on feed, period of time on feed, and scanning day, respectively. Carcass traits, fatty acid concentrations, total lipid percentage, and mRNA enzyme data were analyzed by 1-way ANOVA, with treatment as the independent effect in the model. A repeated measures ANOVA was performed for Warner-Bratzler shear force values of LM in Exp. 2, with the interaction of treatment \times aging included in the model. Agreement between ultrasound and carcass variables was determined using SE of prediction from the PROC REG procedure of SAS. Cellularity data were analyzed with treatment, tissue

Table 1. DNA sequences of the sense (S) and antisense (AS) primers used for synthesis of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), lipoprotein lipase (LPL), and β -actin riboprobes

Gene	Probe sense	Primer sequence ¹	PCR product size, bp
ACC	S	5'-GATGGGCGGGATGGTCTCTTTTC-3'	436
	AS	5'-CCAAGCTTCTAATACGACTCACTATAGGTAGGGCAGGCTCCAGGTGACGATA-3'	
SCD	S	5'-TTCCCGACGTGGCTTTTCTTCT-3'	337
	AS	5'-CCAAGCTTCTAATACGACTCACTATAGGCTCTCGGGGGTTGATGGTCTTGT-3'	
LPL	S	5'-TGTGAAATGCCATGACAAGTC-3'	277
	AS	5'-CCAAGCTTCTAATACGACTCACTATAGTGTGCTATTTGGCCACTATAC-3'	
β -actin	S	5'-GTTCACACTCCTGCCATGTAT-3'	251
	AS	5'-CCAAGCTTCTAATACGACTCACTATAGGTAGCAGAGCTTCTCCTTGATG-3'	

¹Based on sequences reported by Lee et al. (2002) and Bonnet et al. (2001).

(i.m. vs. s.c.), and their 2-way interaction included in the model. Least squares means were separated using the PDIF option and were considered significant at $P \leq 0.05$. Because the experiments were conducted at 2 different times, comparisons between sexes were not made.

RESULTS AND DISCUSSION

Animal Performance and Ultrasound Measurements

The body weight of heifers (Experiment 1) increased over time on feed; however, differences between treatments were observed only at d 80 ($P = 0.07$) and 108 ($P = 0.04$), with SP heifers being heavier than C heifers (Table 2). Average daily gain of SP heifers was faster ($P \leq 0.10$) than C heifers for each period of time on feed except from d 27 to 55. Overall, ADG was 36% faster ($P < 0.01$) for SP than C heifers.

Similar to what was observed in the heifers, BW of steers (Experiment 2) increased over time on feed but did not differ ($P > 0.10$) between treatments during the first 73 d on feed (Table 3). After reimplantation on d 73, BW was greater ($P < 0.05$) on d 105 for SP steers than C steers and tended to be heavier ($P = 0.08$) for SP vs. C steers at d 133. Average daily gain did not

differ ($P > 0.10$) between treatments during the first 73 d of feeding; however, a 40% increase in the rate of growth during the feeding period immediately following reimplantation (d 73 to 105) resulted in a 16% advantage ($P = 0.01$) in ADG for SP over C steers during the entire feeding period. Johnson et al. (1996a) reported that combination implants improved feedlot performance and stimulated carcass protein accretion in steers with the most rapid carcass protein gains observed during the first 40 d after administration. Duckett and Andrae (2001) reported a 19 to 20% increase in ADG for feedlot cattle implanted once or twice with a combination implant. In a study on growing beef cattle grazing bermudagrass, ADG was improved by 11% with zeranol implant and was 10% greater for steers than heifers (Goetsch, et al., 1991).

Ultrasound measurements of LM area, UFT, and UIMF for heifers in Exp. 1 are presented in Table 4. Among the 3 measurements, only ULMA differed between treatments and was greater ($P \leq 0.01$) for SP than C heifers at each measurement time. As expected, measurements of ULMA, UFT, and UIMF increased with days on feed. The SE of prediction showed that live animal ultrasound measurements of ULMA (SE = 5.03 cm²), UFT (SE = 0.17 cm), and UIMF (SE = 0.56%) correlated well with actual carcass measures.

Table 2. Feedlot performance of control (C) and implanted (SP) heifers in Exp. 1

Dependent variable	Item ¹	Treatment		SEM	P-value	
		C (n = 5)	SP (n = 5)			
BW, kg	TOF, d	0	405.52	416.86		
		27	444.07	469.93	10.26	0.24
		55	495.96	525.45	12.49	0.27
		80	525.63	575.52	12.10	0.07
		108	547.22	609.63	13.15	0.04
ADG, kg	Period of TOF, d	0-27	1.43	1.97	0.13	0.08
		27-55	1.85	1.98	0.13	0.62
		55-80	1.19	2.00	0.11	0.01
		80-108	0.77	1.22	0.12	0.10
		0-108	1.31	1.78	0.07	<0.01

¹TOF = Time on feed and refers to the cumulative number of days the animals were on feed, whereas period of TOF refers only to the specified inclusive days the animals were on feed.

Table 3. Feedlot performance of control (C) and implanted (SP) steers in Exp. 2

Dependent variable	Item ¹	Treatment		SEM	P-value	
		C (n = 9)	SP (n = 9)			
BW, kg	TOF, d	0	282.74	275.74		
		28	341.00	338.89	5.56	0.85
		57	390.24	395.79	5.75	0.64
		73	417.06	425.17	5.60	0.48
		105	463.88	490.84	6.27	0.05
		133	486.86	513.57	7.15	0.08
ADG, kg	Period of TOF, d	0-28	2.17	2.26	0.14	0.56
		28-57	1.70	1.96	0.08	0.13
		57-73	1.68	1.84	0.15	0.60
		73-105	1.46	2.05	0.07	<0.01
		105-133	0.82	0.81	0.09	0.96
		0-133	1.53	1.79	0.04	0.01

¹TOF = Time on feed and refers to the cumulative number of days the animals were on feed, whereas period of TOF refers only to the specified inclusive days the animals were on feed.

All 3 steer ultrasound measurements increased with time on feed (Table 5), but none of them were affected ($P > 0.10$) by treatment. Similar to the heifer experiment, SE of prediction for steers showed that live animal ultrasound measurements of ULMA (SE = 4.96 cm²), UFT (SE = 0.18 cm), and UIMF (SE = 1.03%) were accurately measured.

Ultrasound technology has been shown to be highly accurate in estimating live animal composition such as LM area, fat thickness, and intramuscular fat percentage across time on feed when done by a qualified technician. This technology can be a valuable tool for management and in making marketing decisions (Nash et al., 2000; Greiner et al., 2003). The SE of prediction for ultrasound vs. actual carcass values for heifers and steers obtained in this study would meet the requirements for Beef Improvement Federation (BIF, 1997) or Ultrasound Guidelines Council certification (R. Williams, Kansas City, MO, personal communication).

Carcass Attributes

Presented in Table 6 are the slaughter weight and carcass characteristics of control and implanted heifers (Experiments 1) and steers (Experiment 2). Slaughter weight was greater ($P \leq 0.05$) for SP than C in heifers and steers. Heifer HCW tended to be heavier ($P = 0.07$) for SP than C, whereas in steers, HCW was greater ($P = 0.03$) for SP than C. Implanted heifers had a 23% larger ($P < 0.01$) LM area than control heifers. Longissimus muscle area of steers tended to be increased ($P = 0.10$) by implant but to a lesser extent (7%) than was observed in heifers. Heavier carcasses and larger LM areas have been reported previously from steers (Hermesmeier et al., 2000) and heifers (Popp et al., 1997; Mader, 2000) implanted once with a mild or strong combination implant. A review by Dolezal (1997) reported that combination implants used in yearling steers produced the greatest increase in carcass weight and LM area. Mader et al. (1994) also found that reim-

Table 4. Ultrasound measurements of LM area (ULMA), fat thickness (UFT), and i.m. fat percentage (UIMF) of control (C) and implanted (SP) heifers in Exp. 1

Dependent variable	Item ¹	Treatment		SEM	P-value	
		C (n = 5)	SP (n = 5)			
ULMA, cm ²	TOF, d	0	53.73	58.23		
		27	61.54	69.84	1.31	0.01
		55	68.32	79.78	1.77	0.01
		80	76.24	85.69	1.06	<0.01
		108	79.56	92.54	1.11	<0.01
UFT, cm	TOF, d	0	0.73	0.85		
		27	0.87	1.08	0.13	0.45
		55	1.24	1.28	0.12	0.90
		80	1.37	1.38	0.13	0.98
		108	1.57	1.63	0.13	0.82
UIMF, %	TOF, d	0	3.66	4.01		
		27	4.18	4.00	0.31	0.78
		55	4.51	5.19	0.30	0.29
		80	5.26	5.33	0.92	0.92
		108	5.57	5.47	0.32	0.88

¹TOF = Time on feed and refers to the cumulative number of days the animals were on feed.

Table 5. Ultrasound measurements of LM area (ULMA), fat thickness (UFT), and i.m. fat percentage (UIMF) of control (C) and implanted (SP) steers in Exp. 2¹

Dependent variable	Item ²	Treatment		SEM	P-value	
		C (n = 9)	SP (n = 9)			
ULMA, cm ²	TOF, d	0	46.46	44.83		
		28	54.62	52.80	1.06	0.41
		57	61.05	61.65	1.36	0.83
		73	—	—	—	—
		105	70.94	71.48	1.42	0.85
		133	75.33	77.66	1.60	0.48
UFT, cm	TOF, d	0	0.34	0.30		
		28	0.54	0.51	0.03	0.69
		57	0.78	0.71	0.03	0.28
		73	—	—	—	—
		105	1.10	1.05	0.05	0.64
		133	1.25	1.27	0.07	0.86
UIMF, %	TOF, d	0	3.39	3.86		
		28	3.45	4.11	0.20	0.11
		57	4.16	4.23	0.19	0.87
		73	—	—	—	—
		105	5.19	5.42	0.25	0.65
		133	5.28	5.79	0.26	0.34

¹Ultrasound measurements were not collected on d 73.

²TOF = Time on feed and refers to the cumulative number of days the animals were on feed.

planting yearling heifers with a combination implant resulted in heavier carcass weights and increased LM areas compared with control animals. Dressing percent, actual and adjusted fat thickness, and percentage of KPH did not differ ($P > 0.10$) between treatments in Exp. 1 or 2. Other studies have also shown that anabolic implants have little effect on dressing percent (Perry et al., 1991; Johnson et al., 1996a; Scheffler et al., 2003) and subcutaneous fat depth (Herschler et al., 1995; Foutz et al., 1997). Herschler et al. (1995), Foutz et al. (1997), and Scheffler et al. (2003) found no difference in percentage of KPH between nonimplanted and implanted steers. However, other studies have shown that

implants decrease the percentage of KPH in cattle (Johnson et al., 1996a; Duckett et al., 1999; Roeber et al., 2000). Yield grade is influenced by carcass fatness and LM area in relation to carcass weight. Roeber et al. (2000) reported that both LM area and carcass weight are increased by anabolic implants resulting in similar yield grades for implanted and nonimplanted cattle. Data in the current study agree, in that yield grade did not differ ($P > 0.10$) between treatments in heifers or steers. It should be noted however, that 60% of the nonimplanted heifers had USDA yield grades greater than 4, whereas only 20% of the implanted heifers fit into the yield grade 4 category.

Table 6. Slaughter weight (SW) and carcass traits of control (C) and implanted (SP) heifers (Exp. 1) and steers (Exp. 2)

Trait ¹	Heifers				Steers			
	C	SP	SEM	P-value	C	SP	SEM	P-value
No. of animals	5	5			9	9		
SW, kg	524.36	583.33	17.84	0.05	474.31	515.97	11.27	0.02
HCW, kg	338.84	375.62	12.39	0.07	299.07	322.43	6.81	0.03
DP ²	64.62	64.37	0.62	0.78	63.05	62.97	0.47	0.90
AFT, cm	1.80	1.58	0.17	0.36	1.36	1.40	0.06	0.64
LMA, cm ²	70.84	87.10	2.40	<0.01	68.60	73.15	1.85	0.10
KPH, %	1.90	2.10	0.29	0.64	2.67	2.44	0.15	0.31
USDA YG	3.98	3.30	0.31	0.16	3.48	3.46	0.10	0.91
OM ³	162.00	178.00	4.93	0.05	152.22	157.78	4.90	0.44
USDA MS ⁴	566.00	612.00	45.55	0.50	526.11	518.89	36.39	0.89

¹AFT = adjusted fat thickness; LMA = LM area; YG = yield grade; OM = overall maturity; and MS = marbling score.

²DP = dressing percent = (HCW/SW) × 100.

³100 = A⁰⁰ and 200 = B⁰⁰.

⁴500 = Small⁰⁰ and 600 = Modest⁰⁰.

Table 7. Fatty acid composition (% of total fatty acids) and total lipid content of LM from control (C) and implanted (SP) heifers (Exp. 1)

Fatty acid	C	SP	SEM	<i>P</i> -value
C10:0	0.07	0.04	0.020	0.36
C12:0	0.07	0.04	0.012	0.19
C14:0	2.84	2.40	0.207	0.17
C14:1	0.78	0.65	0.060	0.16
C15:0	0.33	0.30	0.015	0.22
C16:0	25.98	24.88	0.808	0.36
C16:1	3.04	2.48	0.161	0.04
C17:0	0.92	0.92	0.042	0.94
C18:0	14.45	15.80	0.452	0.07
C18:1 <i>trans</i>	2.58	2.77	0.272	0.64
C18:1	38.86	39.39	1.180	0.76
C18:2	2.46	2.70	0.163	0.32
C18:3	0.39	0.42	0.039	0.69
CLA, <i>c9t11</i>	0.45	0.45	0.027	0.86
C20:0	0.05	0.07	0.138	0.35
C20:1	0.14	0.23	0.025	0.04
C20:4	0.56	0.43	0.054	0.13
C22:0	0.23	0.14	0.023	0.03
Lipids, %	6.39	6.32	0.434	0.91

Anabolic implant advanced overall maturity by about 10% in heifers ($P < 0.05$) but not in steers ($P > 0.10$). Enhancement of skeletal maturity has been reported in other studies (Belk, 1992; Foutz et al., 1997). The effect of anabolic implant on marbling score has not been consistent among studies. In the current study, marbling score did not differ ($P > 0.10$) between treatments in heifers or steers. Our results are similar to the reports of Johnson et al. (1996a) and Duckett et al. (1997) showing that a single combination implant did not affect marbling in steers and heifers, respectively. However, Herschler et al. (1995) and Mader (2000) documented a reduction in marbling score in steers and heifers implanted once with a mild or strong combination implant. A review by Morgan (1997) showed that steers receiving a mild or strong combination reimplant had a 26-point decrease in marbling score with 24% fewer carcasses grading low Choice. Dolezal (1997) and Roeber et al. (2000) also noted marbling score decreases with readministration of anabolic implant. In the current study, the use of a strong combination implant did not affect quality grade negatively. Perhaps the use of a cattle genotype, which has greater than average potential to marble, is one reason why there was no difference in marbling score between implanted and nonimplanted heifers and steers.

Muscle Lipid Characteristics

Data presented in Table 7 show that total lipid content of LM did not differ ($P = 0.91$) between C and SP heifers (6.39 vs. 6.32%). In addition, total lipid content of LM did not differ ($P = 0.92$) between nonimplanted and implanted steers (5.47 vs. 5.37%). These findings agree with the carcass data of the heifers and steers as well as findings by Gerken et al. (1995) who observed no

Table 8. Warner-Bratzler shear force (WBSF) values of LM from control (C) and implanted (SP) steers (Exp. 2) aged for 1, 3, 7, 14, or 21 d

Aging time, ¹ d	Treatment ¹	
	C	SP
	kg	
1	2.68	3.26
3	2.34	2.69
7	2.14	2.64
14	1.85	2.05
21	1.93	2.01
SEM	0.136	
<i>P</i> -value (treatment × aging time)	0.36	

¹The main effects of treatment and aging time were significant ($P < 0.01$).

impact on longissimus crude fat percentages in Brangus steers implanted once. On the other hand, Foutz et al. (1997) reported that combination implants reduced LM crude fat percentages.

The concentrations of most fatty acids in the LM did not differ ($P > 0.10$) between C and SP heifers (Exp. 1). Only the concentrations of C16:1, C20:1, and C22:0 differed significantly ($P < 0.05$) between nonimplanted and implanted heifers. Among the major fatty acids, the concentration of C18:0 in LM tended to be greater ($P = 0.07$) for SP than C, which is similar to the finding of Duckett et al. (1999). However, this cited study (Duckett et al., 1999) reported that the concentration of C18:1 was reduced with implanting.

Warner-Bratzler Shear Force of LM in Steers

There was no treatment × aging time interaction ($P > 0.10$) on WBSF values of LM (Table 8). Although anabolic implants increased ($P < 0.01$) WBSF values (2.53 vs. 2.19 kg), LM steaks from implanted and nonimplanted steers increased in tenderness over time. Warner-Bratzler shear force values of LM decreased ($P < 0.01$) from d 1 (2.97 kg) to 14 (1.95 kg); however, no further improvement ($P > 0.10$) in tenderness occurred with 21 d (1.97 kg) of aging. Increased WBSF values and decreased tenderness scores in implanted cattle have been reported in other studies (Samber et al., 1996; Platter et al., 2003). Foutz et al. (1997) determined that steaks from steers implanted twice with trenbolone acetate would likely be tougher than steaks from steers administered the same implant only once or 2 estradiol implants. A related study showed that regardless of aging time, top loin steaks from aggressively implanted cattle were tougher than steaks from nonimplanted or conservatively implanted cattle (Morgan, 1997). Other studies that used implants containing trenbolone acetate and estradiol (androgenic and combination) have shown no effect on tenderness of strip loin steaks (Belk and Savell, 1992; Gerken et al., 1995; Barham et al., 2003; Kerth et al., 2003).

Table 9. Adipose cellularity of i.m. and s.c. tissues from control (C) and implanted (SP) heifers in Exp. 1¹

Cellularity trait	Treatment				SEM	P-value (treatment × tissue)
	C		SP			
	i.m.	s.c.	i.m.	s.c.		
Cells per g, × 10 ⁵	6.53 ^b	8.17 ^{ab}	10.42 ^a	6.64 ^b	1.13	0.03
Diameter, μm	80.21	73.31	79.94	77.84	4.80	0.62
Volume, μm ³	593,450	641,434	546,258	708,070	80,569	0.49

^{a,b}Means within a row without a common superscript differ ($P < 0.05$).

¹Cellularity traits were not independently affected ($P > 0.10$) by treatment or tissue.

The decrease in WBSF values over time postmortem indicates that steaks from nonimplanted and implanted cattle benefited from the inherent proteolysis that occurs with aging. A national consumer evaluation of beef tenderness has determined that shear values of <3.0, 3.0 to 4.3, and >4.9 kg would have a 100, 93, and 25% customer satisfaction for beef steak tenderness (Miller et al., 2001). Based on this criterion, all steaks in the current study would have been acceptable for a 100% guaranteed tender product (<3.0 kg of WBSF) after only 1 d of aging.

Adipose Cellularity

Cell diameter distribution did not differ ($P > 0.05$) between nonimplanted and implanted heifers and steers. However, cell diameter distribution differed ($P < 0.05$) by adipose depot in heifers and steers. In heifers, the percentage of s.c. adipocytes in the larger diameter range (>160 μm) was greater ($P < 0.05$) than i.m. (14.7 vs. 5.7%), with a peak diameter (the diameter with the greatest proportion of cells within that subpopulation of cells) of 170 μm. On the other hand, the proportion of i.m. adipocytes was greater ($P \leq 0.01$) than s.c. (29.8 vs. 9.4%) in the middle diameter range (90 to 150 μm). The peak diameter for i.m. adipocytes was 130 μm.

In steers, the percentage distribution of s.c. and i.m. adipocytes followed a similar trend to that observed in the heifers. Adipocytes of s.c. were greater ($P < 0.05$) in proportion in the larger diameter range (>150 μm) than those of i.m. (32.9 vs. 3.5%), with a peak diameter of 160 μm. Conversely, the percentage of i.m. adipocytes was greater ($P < 0.01$) than s.c. (39.6 vs. 17.5%) in the diameter ranges of 50 to 130 μm having a peak diameter

of 110 μm. Numerous studies have observed a hierarchy by depot in adipocyte size with s.c. being greater in diameter than i.m. (Hood and Allen, 1973; Allen, 1976; Schiavetta et al., 1990; Mendizabal et al., 1999). Gilbert et al. (2003) reported peak diameters of approximately 150 and 100 μm for s.c. and i.m. adipocytes, respectively. May et al. (1994) reported peak diameters of 180 and 140 μm for s.c. and i.m. adipocytes, respectively, in Angus and Wagyu steers. Our results, particularly those from the steers, closely resemble the data of Gilbert et al. (2003) but were smaller compared with the values reported by May et al. (1994).

Adipose tissue can enlarge by hyperplasia (cell proliferation) or hypertrophy (cell enlargement through lipid accumulation). As fat is deposited in cattle, a population of cells will accumulate lipid, increasing in diameter and volume. Once this population of cells attains a certain size due to hypertrophy, another population of smaller adipocytes becomes apparent resulting in a biphasic diameter distribution (Allen, 1976). The increase in adipocyte number may be due to preadipocytes filling with lipid and becoming large enough to be counted using the Coulter counter or through actual differentiation or proliferation of newly stimulated adipocytes (Hood, 1982). The occurrence of smaller adipocytes suggests reinitiation of hyperplasia or differentiation of preadipocytes (Allen, 1976). The exact cell size when this recruitment occurs is unknown for s.c. and i.m. adipose tissue. Because of a discrepancy between s.c. and i.m. tissue as to when biphasic cell distribution is displayed, Allen (1976) and Schoonmaker et al. (2004) have reported that hyperplasia in s.c. tissue may be triggered at a larger adipocyte size compared with i.m. tissue.

Table 10. Adipose cellularity of i.m. and s.c. tissues from control (C) and implanted (SP) steers in Exp. 2¹

Cellularity trait	Treatment			Tissue			SEM
	C	SP	P-value	i.m.	s.c.	P-value	
	Cells per g, × 10 ⁵	3.91	4.78	0.07	5.51	3.19	
Diameter, μm	95.48	94.93	0.80	82.63	107.78	<0.01	
Volume, μm ³	854,917	812,274	0.33	489,452	1,177,739	<0.01	

¹There was no treatment × tissue interaction ($P > 0.10$) for any of the cellularity traits.

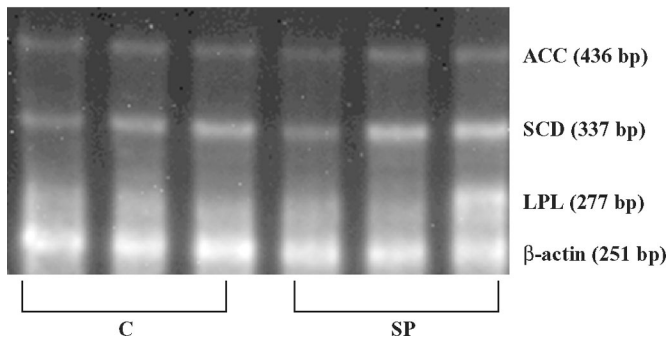


Figure 1. Ribonuclease protection assay (RPA) of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), lipoprotein lipase (LPL), and β -actin expression in i.m. adipose tissue of control (C) and implanted (SP) heifers in Exp. 1.

Data on cellularity of i.m. and s.c. adipose tissues in heifers and steers are presented in Tables 9 and 10, respectively. In the heifers, there was a treatment \times tissue interaction ($P = 0.03$) for the number of cells per gram of dissected adipose tissue. The number of i.m. adipocytes was greater ($P < 0.05$) for SP than C and was greater ($P < 0.05$) than s.c. among SP heifers. Average cell diameter and volume did not differ ($P > 0.10$) between treatments and tissues.

In the steers, the number of cells per gram of dissected adipose tissue tended to be greater ($P = 0.07$) with implanting. Additionally, i.m. tissue had greater ($P < 0.01$) counts per gram of tissue than s.c. Average cell diameter and volume did not differ ($P > 0.10$) between treatments, but both cellularity traits were larger ($P < 0.01$) for s.c. than i.m. No data have been published previously to show the effect of hormone implants on adipose cellularity. May et al. (1994) reported that i.m. adipose contained more cells per gram of tissue with a smaller mean cell diameter and volume relative to s.c. adipose. Marbling is thought of as a late-maturing fat depot that is not fully developed at slaughter (Hood and Allen, 1973; Cianzio et al., 1985; May et al., 1994). Duckett and Andrae (2001) found an inverse relationship between marbling score and LM area, a consequence of dilution of i.m. fat in a larger LM area. In this study, the modest (steer) to significant (heifer) increase in LM area of implanted animals was accompanied by a corresponding increase in the number of i.m. adipocytes. This result along with the lack of differences in total lipid contents may explain why implanting did not impact marbling score between treatments in either experiment.

Enzyme Expression

As alluded to earlier, anabolic implants may have an indirect effect on i.m. lipid deposition. Fat accretion is the balance between fat synthesis (lipogenesis) and breakdown (fatty acid oxidation/lipolysis). Three key

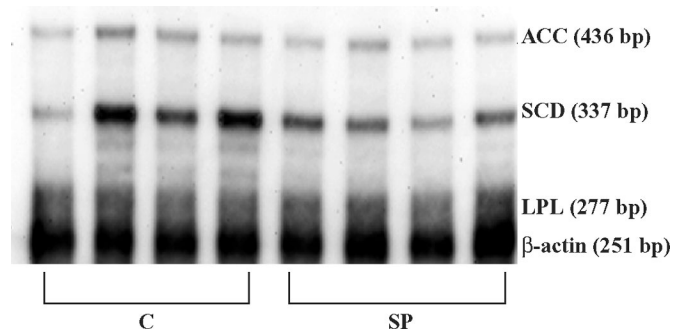


Figure 2. Ribonuclease protection assay (RPA) of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), lipoprotein lipase (LPL), and β -actin expression in i.m. adipose tissue of control (C) and implanted (SP) steers in Exp. 2.

enzymes involved in the uptake and biosynthesis of fatty acids are LPL, ACC, and SCD). Lipoprotein lipase catalyzes the hydrolysis of triglycerides from circulating lipoprotein particles and thus provides free fatty acids to adipose tissue (Auwerx et al., 1992). The rate-limiting enzyme of fatty acid synthesis or lipogenesis is ACC (Abu-Elheiga et al., 2001). In this initial biotin-dependent step, acetyl CoA is converted to malonyl-CoA, which is the substrate for fatty acid synthesis. Fatty acid composition of lipids in adipose tissues is greatly regulated by SCD. Stearoyl coenzyme A desaturase catalyzes the rate-limiting step in the biosynthesis of MUFA by inserting a *cis*-double bond in the fatty acyl-CoA substrate Δ^9 position (Kim and Ntambi, 1999).

The concentrations of mRNA for ACC, SCD and LPL from i.m. adipose tissue of nonimplanted and implanted heifers (Figure 1) and steers (Figure 2) were analyzed by ribonuclease protection assay. Densitometric quantification of mRNA abundance of the lipogenic enzymes (ACC, SCD, and LPL) in i.m. adipose tissue did not

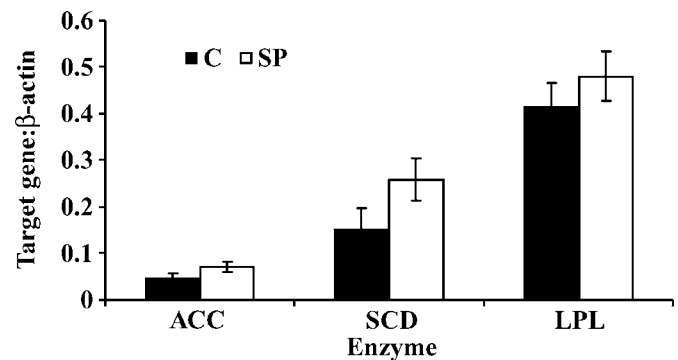


Figure 3. Densitometric quantification of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), and lipoprotein lipase (LPL) mRNA from i.m. adipose tissue of control (C) and implanted (SP) heifers in Exp. 1. Data are expressed as means \pm SE of 5 animals in each treatment.

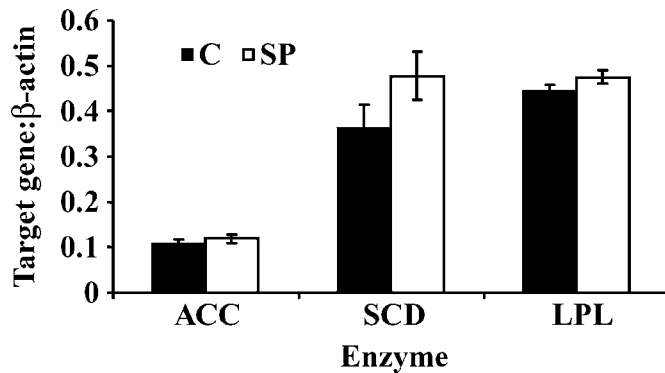


Figure 4. Densitometric quantification of acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), and lipoprotein lipase (LPL) mRNA from i.m. adipose tissue of control (C) and implanted (SP) steers in Exp. 2. Data are expressed as means \pm SE of 5 animals in each treatment.

differ ($P > 0.05$) between treatments in heifers (Figure 3) or steers (Figure 4). In another study, SCD enzyme activity in interfascicular and s.c. adipose tissues was not affected by feeding different levels of whole cottonseed or different grains to finishing steers (Archibeque et al., 2005). The lack of difference between treatments in the expression of lipogenic enzymes may likewise explain the absence of differences in the concentrations of major fatty acids in the LM of heifers. Data on mRNA concentrations of all 3 lipogenic enzymes and the preceding data on i.m. adipose cellularity and marbling score indicate that anabolic implants did not appear to have a direct effect on i.m. lipid deposition in this study.

IMPLICATIONS

Anabolic implants enhance animal performance and carcass protein accretion through an increase in longissimus muscle area. However, implants did not alter longissimus muscle intramuscular lipid deposition, measured by marbling score, total lipid content, fatty acid content, adipocyte cellularity, or lipogenic enzymes expression. Collectively, these data suggest that anabolic implants do not have a direct effect on intramuscular lipid deposition, particularly in cattle with a high genetic propensity to deposit intramuscular fat.

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