Effects of implant programs on performance, carcass characteristics, and lipogenic gene expression in Holstein steers

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Abstract

Nineteen Holstein steers were randomly assigned to one of four treatments; no implant (CON); 36 mg zeranol (Ralgro) on d 0 and 20 mg estradiol benzoate plus 200 mg progesterone (Synovex S) on d 84 and 169 (RSS); Ralgro on d 0, Synovex S on d 84, and 28 mg estradiol benzoate plus 200 mg trenbolone acetate (Synovex-Plus) on d 168 (RSP); or Ralgro on d 0, and Synovex-Plus on d 84 and 168 (RPP). Subcutaneous adipose biopsies were collected before implanting (d -14) and on d 97, 182 and at slaughter for measurement of mRNA concentrations of fatty acid synthetase (FAS) and acetyl CoA carboxylase (ACC). Implanted steers had greater average daily gain, dry matter intake, final body weight, hot carcass weight and longissimus muscle area than controls. No differences were observed in feed conversion ratio (FCR), dressing percentage, 12th rib backfat, kidney pelvic and heart fat, marbling score, yield grade or Warner-Bratzler shear force values. Treatment did not affect serum metabolite concentration. Real Time PCR analysis of subcutaneous adipose mRNA concentrations indicated implants decreased ACC and FAS expression on d 97. ACC expression was increased significantly for RPP compared to RSP on d 182 and increased for the average of RSP and RPP compared to RSS on d 259, whereas FAS expression was not affected on d 182 or 259. Results suggest that lipogenic gene expression is affected by anabolic implants, particularly early in the feeding period and may partially explain how implants affect carcass characteristics, albeit a limited number of steers were used.

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Introduction

It is widely accepted in the beef industry that anabolic implants improve live performance compared with non-implanted controls (Bartle *et al*., 1992; Duckett *et al.*, 1997; Bruns *et al.*, 2005). However, using anabolic implants has caused concerns in the packing industry about unfavourable effects on carcass quality. The most emphasized detrimental effect is a decrease in quality grade due to decreased marbling score (MS; Roeber *et al*., 2000; Platter *et al*., 2003). In addition, repetitive and/or aggressive implant programs have been reported to affect palatability and beef quality (Roeber *et al.*, 2000; Platter *et al.*, 2003). However, a recent review by Nichols *et al*. (2002) suggested that there is little or no effect of implants on beef tenderness.

Most research attempting to elucidate the mechanisms of action of anabolic implants has focused on muscle and protein metabolism with little work focusing on adipose physiology. Acetyl-CoA carboxylase (ACC) and fatty acid synthetase (FAS) are the two key enzymes responsible for fatty acid synthesis and reduced quantities of these proteins could reduce *de novo* fatty acid synthesis and may decrease intramuscular adipose deposition. Oestrogen, progesterone, and androgen (hormones commonly found in implants) receptors have been identified in adipocytes from several species and thus their respective ligand could alter the transcription of several lipogenic genes (Mayes & Watson, 2004). Therefore, implants containing these steroid hormones may directly regulate fatty acid synthesis at the genomic level.

Our objectives were to evaluate the effects of anabolic implant programs, commonly implemented in the United States feedlot industry, on ACC and FAS gene expression. Our hypothesis was that more aggressive implant programs would have a profound effect on MS and carcass adipose depots while simultaneously decreasing ACC and FAS gene expression.

Materials and Methods

The University of Arizona Institutional Animal Care and Use Committee reviewed and approved this protocol.

Twenty Holstein steers [body weight (BW) 190 ± 22 kg] were purchased from a local producer, delivered to the University of Arizona Feedlot (Tucson), placed in a 26.6 x 56.6 m pen and fed an 80% concentrate diet for a 30 d adaptation period to mimic that of the calf feeding operation. Following this adjustment period, diets were increased to an 86% concentrate diet (Table 1) in a stepwise manner. Steers were treated for internal and external parasites (Ivomec; Merical, Duluth, G.A.), vaccinated with infectious bovine rhinotracheitis, parainfluenza-3, bovine viral diarrhoea, bovine respiratory synsytial virus plus pasteurella haemolytica (Pyramid 4 + Presponse SQ, Fort Dodge Animal Health, Fort Dodge, I.A.) and a clostridial preparation (Ultra Choice 8; Pfizer Animal Health, Exton, P.A.). Nineteen steers (BW = 182 kg) were then randomly assigned to one of four treatments and randomly allocated to individual, partially shaded, soil-surfaced pens (2.5 x 6 m) with an individual water source and feed bunk. Treatments included: 1) no implant (CON; n = 5); 2) implanted with 36 mg of zeranol (Ralgro; Schering-Plough Animal Health) on d 0 followed by an implant of 20 mg of estradiol benzoate (E) + 200 mg of progesterone (Synovex-S; Fort Dodge Animal Health) d 84 and 168 (RSS; n = 5); 3) implanted with Ralgro on d 0 followed by an implant of Synovex-S on d 84 and 28 mg E + 200 mg trenbolone acetate (TBA; Synovex Plus; Fort Dodge Animal Health) on d 168 (RSP; n = 5); 4) an implant with Ralgro on d 0 followed by an implant with Synovex-Plus on d 84 and 168 (RPP; n = 4). Prior to use and between each animal, the implant gun was immersed in Nolvasan (Fort Dodge Animal Health) and dried before implant was inserted. After each implant, the ear was palpated to ensure all pellets were inserted and securely in place.

Table 1 Ingredient and chemical composition of diet

	Diet composition (g/kg DM)
Ingredient	
Steam flaked maize	675
Sorghum sudangrass hay	140
Soyabean meal	62.5
Molasses	50
Tallow	40
Urea	7.5
Supplement[a]	25
Chemical Composition	
Dry matter	877
Ash	140
Crude protein	154
Acid detergent fibre	79

[a] Supplement composition (DM basis): limestone, 47.059%; dicalcium phosphate, 1.036%; potassium chloride, 8.000%; magnesium oxide, 3.448%; ammonium sulphate, 6.667%; salt, 12.000%; cobalt carbonate, 0.002%; copper sulphate, 0.157%; iron sulphate, 0.133%; calcium iodate, 0.003%; manganese sulphate, 0.500%; selenium premix (0.16%), 0.125%; zinc sulphate, 0.845%; vitamin A (30,000 IU/g), 0.264%; vitamin E (500 IU/g) 0.540%; Rumensin-80, 0.675%; Tylan 40, 0.450%; ground maize, 18.096%.

Steers were fed (*ad libitum*) once daily at 06:30 and sprayed with a fly repellent (Co-Ral, Bayer Healthcare LLC, Shawnee Mission, K.S.) on an as-needed basis. Water troughs and feed bunks were cleaned weekly and ort samples were collected individually weighed (Ohaus Champ II Bench Scale, Ohaus Corporation, Pine Brook, N.J.) and recorded before being pooled and sampled for DM analysis. Steers were weighed every 28 d prior to feeding, and pens were mechanically scraped to prevent manure build up. On d 259, steers were humanely slaughtered via stunning and exsanguination at the University of Arizona Meat Laboratory. Hot carcass weight (HCW) was recorded and loin muscle (LM) area, adjusted backfat (BF,

12th rib) thickness, kidney, pelvic and heart fat (KPH), firmness, colour, texture, MS and skeletal maturity were measured and collected according to United States Department of Agriculture guidelines (USDA 1997) after a 48-h chill period. Carcasses were processed and two, 1.27 cm steaks were cut from the 12th rib section for Warner-Bratzler shear force (WBSF) value analysis. Steaks were labelled and frozen (-20 °C) for later analysis.

Adipose tissue biopsies were taken on d -14, 97, and 182 and a final sample was taken at slaughter. Adipose tissue was obtained from the area dorsal to the hipbone. The area was shaved, cleaned with betadine and 70% isopropyl alcohol and anesthetized by local infiltration of 5 to 10 mL of 2% lidocaine (lidocaine hydrochloride, injectable, 2%; Vedco Inc., St. Joseph, M.O.). A 3.8 cm incision was made to a depth to expose adipose tissue and approximately 1.5 g of adipose tissue was excised. The biopsy site was flushed with 70% isopropyl alcohol and the incision site was sutured using sterile general closure (70 cm, 2-0 coated vicryl suture; Ethicon, Somerville, N.J.). The site was sterilized further with 70% isopropyl alcohol and wiped dry with cotton gauze (Kendall Curity Gauze Sponges, Tyco Healthcare Group, Mansfield, M.A.). Alushield Aerosol Bandage (IDEAL Animal Health, Lexington, K.Y.) was sprayed directly over the incision followed by a ring of Blu-Kote (H.W. Naylor Co. Inc., Morris, N.Y.) around the previous spray to prevent bacterial or fungal infection. At slaughter, biopsy site adipose samples were collected from each animal and placed in a 15 mL polypropylene flatcap tube (Corning Inc.). All biopsy samples were immediately placed in a polypropylene flat cap tube (Corning Inc., Corning, N.Y.) and flash frozen in liquid nitrogen for transport to the University of Arizona Ruminant Nutrition Laboratory and stored at –80 °C for later analysis.

Venous blood samples were collected prior to feeding from the jugular vein using vacuum tubes (Vacutainer; Becton Dickinson & Co., Franklin Lakes, N.J.) on d -14, 0 and every 28 d thereafter until the completion of the study. Whole blood was transferred to the University of Arizona Ruminant Nutrition laboratory and serum was separated, via centrifugation at 1,000 x g for 20 min, harvested and stored (-20 °C) for later analysis.

Feed and ort samples were subjected to dry matter (DM) analysis (oven drying at 55 °C until no further weight loss), and feed samples were ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, P.A.) to pass a 1 mm screen and analyzed for crude protein (CP: % N x 6.25; LECO Corporation, St. Joseph, M.I.), acid detergent fibre (ADF: determined without adjustment for residual ash; ANKOM Technol. Corp., Fairport, N.Y.) and ash (combusted 6 h in muffle furnace at 500 °C).

Serum samples were analyzed for urea nitrogen (SUN**;** TECO Diagnostics, Anaheim, C.A. 92807), glucose (Glucose C2, Wako Chemicals, Richmond, V.A.), and non-esterified fatty acids (NEFA: NEFA C, Wako Chemicals, Richmond, V.A.) using colorimetric methods as specified in kit protocols and validated for use in our laboratory. The inter- and intra-assay coefficients were 4.83, 4.90; 11.18, 11.06; and 10.25, 10.18 for glucose, NEFA and SUN, respectively.

For WBSF analysis, steaks were allowed to thaw to room temperature. Each steak was cooked on an open-hearth grill until internal temperature reached 35 °C turned over and allowed to continue cooking until the internal temperature reached 70 °C. Steaks were allowed to return to room temperature and eight, 2.54 cm core samples, per steak, were removed and tested for shear force values. High and low scores were eliminated and the remaining six were used to calculate a mean value.

For adipose gene expression analysis, adipose tissue (0.5 g) was homogenized (Polytron, Brinkmann Instruments, Inc., Westbury, N.Y.) and total RNA was extracted with TRIzol (Invitrogen; Carlsbad, C.A.) according to manufacturer's guidelines. A chloroform extraction was then performed followed by an isopropyl/0.8 M Na_3 Citrate + 1.2 M NaCl precipitation and an ethanol wash. Pelleted RNA was resuspended in 50 μL DEPC H_2O. Sample concentration was analyzed by photospectrometry using a 1:100 dilution. RNA was normalized to 0.5 μg concentration and DNase treated (DNase I – Amplification grade, Invitrogen) and cDNA created using Super Script ™ III First-Stand Synthesis System for RT-PCR (Invitrogen) according to product guidelines. Real-time primers were created using published sequences and selected based on visualization on a 1.5% agarose gel (Table 2). Polymerase chain reaction products were sequenced to ensure primer specificity.

Samples were randomly allocated to plate wells and ran in triplicate alongside triplicate standard curves of the gene of interest and duplicate standard curves of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Two cDNA sample pools were run in duplicate on every plate with GAPDH as the primer. These

Table 2 Real time primer sequences

Primer	Sequence (5' to 3')	CDS [a]	Length [b]	EMBL ac. No.
ACCf [c]	GGGAAGGAAGAAGGACTTGG	5699 – 5720	166	NM174224 (bovine)
ACCr [d]	CGACCTGGATGGTTCTCTGT	5839 – 5864		
GAPDHf [e]	CATTGACCTTCACTACATGGT	164 – 184	236	AB098985 (bovine)
GAPDHr [f]	ACCCTTCAAGTGAGCCCCAG	381 – 400		
FASf [g]	GGAGGACGCTTTCCGTTACA	15480 – 15499	52	AF285607 (bovine)
FASr [h]	TGACCACTTTGCCGATGTGT	15511 – 15532		

[a] Coding sequence; [b] Product length; [c] Acetyl CoA carboxylase forward primer;
[d] Acetyl CoA carboxylase reverse primer; [e] Glyceraldehyde-3-phosphate dehydrogenase forward primer;
[f] Glyceraldehyde-3-phosphate dehydrogenase reverse primer; [g] Fatty acid synthetase forward primer;
[h] Fatty acid synthetase reverse primer.

pools served as inter-plate variables. Another pooled cDNA sample was run in duplicate on every plate with the gene of interest as the primer. Ninety-six well Optical Reaction Plates with Barcode (Applied Biosystems, Foster City, C.A.), BioRad iCycler iQ Optical Tape (Bio-Rad Laboratories, Hercules, C.A.), and iTaq SYBR Green Supermix with Rox (Bio-Rad Laboratories) were used on every plate to insure continuity.

Genomic Analysis and Technology Core facilities at the University of Arizona were used to run Real-time programs on the ABI PRISM 7000 Sequence Detection System (Applied Biosystems). ABI PRISM 7000 Sequence Detection System Software (Applied Biosystems) was used to visualize and interpret the data. Real-time PCR conditions for GAPDH and FAS were 1 cycle at 95 °C for 10 min for denaturation followed by 40 cycles at 95 °C for 15 sec and 58 °C for 1 min for annealing. ACC conditions were identical except for an annealing temperature of 60 °C. Disassociation curves were run on every plate to insure single product amplification per primer set. A sample volume of 20 μL was identical for each sample well on every plate. Background baseline cycles were 6 and 15 for all plates. After each run, primer efficiencies were calculated using the equation:

$$\text{Efficiency} = 1 - 10^{(-1/\text{ slope of the standard curve})}$$

Glyceraldehyde-3-phosphate dehydrogenase, ACC and FAS efficiencies were 98, 97, and 98% averaging all triplicates across all plates. Interplate CV-values across all plates for GAPDH Pool 1, GAPDH Pool 2, ACC and FAS were 6.0, 3.1, 2.1 and 1.3%, respectively. Standard deviation and CV values were calculated for cycle threshold values. If an outlier in the triplicate was present, it was removed and duplicate values were used. The coefficients of variation of all values were lower than 1.0%. Glyceraldehyde-3-phosphate dehydrogenase values were statistically analyzed using SAS (2004) with a model that included treatment and month to insure no difference ($P = 0.39$) due to treatment, thus insuring a proper choice for an endogenous reference gene. Delta ΔcT values were used for analysis and obtained by the equation:

$$\Delta cT = cT \text{ (target gene)} - cT \text{ (endogenous reference gene)}$$

When ΔcT values increase this indicates a corresponding decrease in gene expression and *vice versa*.

All data sets were statistically analyzed using the MIXED procedure of SAS (2004). For performance and carcass characteristics, the model included effects for treatment and steer was used as a random effect. Serum metabolites were analyzed with repeated measures analysis, and the model included effects for treatment, sampling date and treatment by sampling date interaction as fixed effects. Gene expression data were analyzed with a model that included effects for treatment, sampling date and treatment by sampling date interaction as fixed effects and animal was used as a random effect and sampling date as a repeated factor. Auto regressive of order 1 was used for covariance structure. A Treatment x sampling date interaction was observed for ACC ($P < 0.02$) and data were analyzed within sampling date using the MIXED procedure of SAS (2004). Within sampling date, the model included effects for treatment and covariance. Individual FA data were analyzed using the MIXED procedure of SAS (2004) with a model including treatment, tissue (s.c. *vs*. abdominal) and treatment by tissue interaction. Steer was used as a random effect. Preplanned

contrasts for all data included 1) control *vs*. the average for implants, 2) RSS *vs.* the average of RSP and RPP, and 3) RSP *vs*. RPP.

Results

As expected, implanted cattle performed better than non-implanted controls (Table 3). Final BW, average daily gain (ADG), and dry matter intake (DMI) were greater (P <0.01) for implanted steers than for control steers, but no treatment differences (P >0.54) were observed between the various implant strategies for these variables. Implants increased ADG by approximately 23% and DMI by 18%. Gain : feed did not differ between implanted and control steers or as a result of implant strategy (P >0.19).

All carcasses fell into the A maturity category, and no instances of dark cutters was observed (data not shown). Hot carcass weight and LM area were increased (P <0.01) in implanted steers compared to controls, but no differences (P >0.56) were observed as a result of implant strategy. No differences (P >0.10) were observed between treatments for DP, adjusted BF, KPH, MS, YG or WBSF (Table 3).

Table 3 Mean (± s.e.) of live performance and carcass characteristics of Holstein steers on varying implant programs

Item	Treatment [a,b]			
	C ON	RSS	RSP	RPP
No. of steers	5	5	5	4
Initial BW, kg [c]	184 ± 6.2	188 ± 6.2	189 ± 6.2	191 ± 6.2
Final BW, kg [d]	509 ± 12.8	585 ± 12.8	584 ± 12.8	576 ± 12.8
ADG, kg d 0 to end [d]	1.29 ± 0.15	1.57 ± 0.15	1.57 ± 0.15	1.53 ± 0.15
DMI, kg/d d 0 to end [d]	8.1 ± 0.35	9.6 ± 0.35	9.4 ± 0.35	9.6 ± 0.35
G:F d 0 to end [c]	0.16 ± > 0.01	0.16 ± > 0.01	0.17 ± > 0.01	0.16 ± > 0.01
HCW, kg	292 ± 7.5	340 ± 7.5	337 ± 7.5	334 ± 7.5
LM area, cm^{2}; [d]	64.4 ± 2.8	76.1 ± 2.8	73.9 ± 2.8	76.1 ± 2.8
Dressing %	57.5 ± 0.01	58.0 ± 0.01	58.1 ± 0.01	58.0 ± 0.01
Adjusted fat thickness, cm [c]	0.66 ± 0.14	0.89 ± 0.14	0.66 ± 0.14	0.70 ± 0.14
KPH, % [b]	5.1 ± 0.39	5.1 ± 0.39	4.2 ± 0.39	4.4 ± 0.39
Marbling score [c, e]	554 ± 54.0	644 ± 54.0	540 ± 54.0	643 ± 54.0
Yield grade [c]	3.4 ± 0.25	3.5 ± 0.25	3.2 ± 0.25	3.1 ± 0.25
Warner-Bratzler shear force, kg [c]	1.98 ± 0.31	2.14 ± 0.31	2.25 ± 0.31	2.52 ± 0.31

[a] C ON = no implant; RSS = Ralgro on d 0, Synovex S on d 84 and Synovex S on d 168; RSP = Ralgro on d 0, Synovex S on d 84 and Synovex Plus on d 168; RPP = Ralgro on d 0, Synovex Plus on d 84 and Synovex Plus on d 168.

[b] Contrasts evaluated 1 = CON *vs*. implants; 2 = RSP *vs*. RPP; 3 = RSS *vs*. the avg. of RSP and RPP.

[c] Not significant; [d] Contrast 1 (P <0.05).

[e] Marbling scores: 500 = small; 600 = modest; 700 = moderate.

No treatment x sampling date interactions (P >0.28) were noted for serum metabolites (Table 4). Averaged across all sampling days, no differences (P >0.13) were observed for glucose, NEFA or SUN concentrations in implanted steers compared to control steers or as a result of implant strategy.

Acetyl CoA carboxylase and FAS data are presented in Table 5. Subcutaneous ACC and FAS gene expression were decreased (P <0.01) by anabolic implants at d 97. Acetyl CoA carboxylase expression was increased (P <0.02) on d 182 for RPP compared with RSP and also increased (P <0.03) on d 259 for the average of RSP and RPP compared with RSS. During these same time points FAS did not differ (P >0.15).

Table 4 Mean (± s.e.) of serum metabolites of Holstein steers on varying implant programs[a]

Item	Treatment[b,c]			
	C ON	RSS	RSP	RPP
Glucose, mg/dL[d]	99.9 ± 3.8	95.4 ± 3.8	101.3 ± 3.8	99.6 ± 3.8
NEFA, μEq/L[d]	268.5 ± 21.5	274.2 ± 21.5	312.6 ± 21.5	312.2 ± 21.5
Serum urea nitrogen, mg/dL[d]	5.2 ± 0.2	5.1 ± 0.2	5.3 ± 0.2	4.9 ± 0.2

[a] No treatment x day interactions (P >0.28); therefore, data were averaged across all sampling days.
[b] C ON = no implant; RSS = Ralgro on d 0, Synovex S on d 84 and Synovex S on d 168; RSP = Ralgro on d 0, Synovex S on d 84 and Synovex Plus on d 168; RPP = Ralgro on d 0, Synovex Plus on d 84 and Synovex Plus on d 168.
[c] Contrasts evaluated 1 = CON *vs*. implants; 2 = RSP *vs*. RPP; 3 = RSS *vs*. the avg. of RSP and RPP.
[d] Not Significant.

Table 5 Mean (± s.e.) ΔcT values of acetyl CoA carboxylase (ACC) and fatty acid synthetase (FAS) gene expression in subcutaneous adipose tissue of Holstein steers receiving varying implant treatments[a, b]

Item	Treatment[c,d]			
	C ON	RSS	RSP	RPP
ACC				
d 97[e]	2.06 ± 0.56	4.48 ± 0.56	3.30 ± 0.56	4.25 ± 0.56
d 182[f]	3.20 ± 0.96	3.26 ± 0.96	5.96 ± 0.96	2.35 ± 0.96
d 259[g]	3.62 ± 1.67	8.74 ± 1.67	4.50 ± 1.67	2.91 ± 1.67
FAS				
d 97[e]	0.30 ± 0.97	3.30 ± 0.97	3.53 ± 0.97	4.35 ± 0.97
d 182[h]	3.17 ± 1.72	2.73 ± 1.72	5.06 ± 1.72	3.40 ± 1.72
d 182[h]	5.34 ± 1.73	6.67 ± 1.73	2.38 ± 1.73	5.06 ± 1.73

[a] Delta cT values are presented in the table and were used for analysis. Values were obtained by the equation: ΔcT = cT(target gene) – cT(endogenous reference gene). Increasing change in gene expression is shown as a decrease in numerical value.
[b] Treatment x sampling date interactions were observed for both ACC (P <0.02) and FAS (P <0.08) and data were analyzed within sampling date.
[c] C ON = no implant; RSS = Ralgro on d 0, Synovex S on d 84 and Synovex S on d 168; RSP = Ralgro on d 0, Synovex S on d 84 and Synovex Plus on d 168; RPP = Ralgro on d 0, Synovex Plus on d 84 and Synovex Plus on d 168.
[d] Contrasts evaluated 1 = CON *vs*. implants; 2 = RSP *vs*. RPP; 3 = RSS *vs*. the avg. of RSP and RPP.
[e] Contrast 1 (P <0.05); [f] Contrast 2 (P <0.05); [g] Contrast 3 (P <0.05); [h] Not significant.

Discussion

Animal performance results agree with Perry *et al*. (1991), Samber *et al*. (1996) and Guiroy *et al*. (2002), and indicating implanted steers had greater ADG compared to non-implanted control animals. Additionally, our results agree with Scheffler *et al.* (2003) who reported Holstein steers implanted twice and three times with E + TBA (24 mg E + 120 mg TBA) had greater ADG when compared to Holstein steers implanted singly or not implanted. However, we were somewhat surprised that the E + TBA implants did not improve performance over the progesterone-E implant. For example, Foutz *et al*. (1997) showed E + TBA implanted steers gained more rapidly than non-implanted or E + progesterone implanted steers. Reasons for the discrepancy may be related to the small number of animals. In addition, implanted steers in this trial were not more efficient than CON steers, which differ from previous studies where implanted animals had enhanced feed efficiency (Samber *et al*., 1996; Foutz *et al*., 1997). Final BW data agrees with previous

reports where Holstein steers implanted 2 or 3 times showed greater final BW than control steers (Scheffler *et al*., 2003).

Except for a few instances, our data are in general agreement with previous reports on the influence of anabolic implants on carcass characteristics. Not surprisingly, because of performance advantages, implants increased HCW and LM area. This is supported by previous studies, which concluded that implants have a marked enhancement on carcass weight (Hermesmeyer *et al*., 2000; Roeber *et al*., 2000; Platter *et al*., 2003) and LM area (Platter *et al*., 2003). Dressing percentage in the current study was not affected by implantation and this differs with Herschler *et al*. (1995) who reported Synovex-S implants increased DP. Because implants increase muscling and have been reported to decrease organ weights (Hutcheson *et al*., 1997), DP would be expected to be lowered. However, our results agree with Samber *et al*. (1996) who reported no difference among implant treatments and Scheffler *et al*. (2003), which specifically focused on Holstein steers. Our skeletal maturity data is opposed to reports of implants advancing bone maturity with subsequent implantation (Scheffler *et al*., 2003). However, our data is in partial agreement with Foutz *et al*. (1997) who reported that while implanted steers grade-A maturity, they have more advanced maturation when compared to control steers. The lack of differences in fat thickness is in agreement with several studies which reported no differences between control and implanted crossbred cattle (Samber *et al*., 1996; Foutz *et al*., 1997; Platter *et al*., 2003) or Holstein steers (Apple *et al*., 1991; Scheffler *et al*., 2003). Our results for KPH were in agreement with studies finding no differences between treatments (Samber *et al*., 1996; Foutz *et al*., 1997) especially with those studies that used Holstein steers (Apple *et al*., 1991; Scheffler *et al*., 2003). However, trial results are in conflict with Platter *et al*. (2003) who observed a decrease in KPH values in anabolically implanted animals. Additionally, in our study, steers implanted with Synovex-S did not respond similarly to reports of Synovex-S dosed steers having reduced KPH scores compared with control steers (Herschler *et al*., 1995).

Marbling score data from this study are in conflict with several studies, which found decreased MS with the use of implants. Specifically, TBA implanted steers did not respond similarly to Foutz *et al*. (1997) who reported that TBA implanted crossbred steers had lower MS compared to E + P (20 mg E + 140 mg P) implanted and control steers. Furthermore, TBA treated steers differed from Herlschler *et al*. (1995) implant data where steers dosed 1 : 5 and 1 : 10, E : TBA had lower MS than non-implanted steers. All steers had three successive implants and did not react in the same way as steers from Samber *et al.* (1996) who reported lower percent choice and prime carcass grades. Lastly, steers implanted with Synovex-S in Herschler *et al*. (1995) had a decreased MS and quality grade compared to untreated steers, which differs from steers dosed similarly in this study. However, it was not our objective to elucidate any industry focused implant effects on quality grades as seen by our low animal numbers. Contrary to Foutz *et al*. (1997), who reported steers implanted with TBA received lower YG scores compared to non-implanted or steers implanted with E + P, our data show no treatment differences. This supports Scheffler *et al*. (2003) findings of no effect of implant treatment. Nichols *et al.* (2002) reviewed 19 publications and found that Warner-Bratzler shear force values did not differ between implanted and non-implanted cattle when multiple treatments were examined. Additionally, Apple *et al*. (1991) saw no difference between implanted and control Holstein steers in WBSF. However, our results are in contrast to previous studies, which focused on the adverse effects of implants on meat tenderness (Samber *et al*., 1996; Roeber *et al*., 2000). These authors reported that anabolic growth promotants have unfavourable effects on meat tenderness, shear force values, and beef grade factors. In this study 17 of the 19 steers graded Choice or better, therefore it is not surprising that we failed to detect a difference in WBSF.

Initially we theorized SUN levels would decrease according to implant program aggressiveness due to increased rates of protein accretion and both Hongerholt *et al*. (1992) and Preston *et al.* (1995) reported anabolic implants decreased plasma urea nitrogen concentrations. However, our results did not agree with either report, since no differences in SUN were noted among treatments. This is not completely surprising since animals were in a well-fed state depositing both adipose and muscle tissues.

Changes in ACC and FAS gene expression could alter *de novo* lipogenesis and modify adipose tissue deposition. Adipocyte receptors for oestrogen, progesterone and androgens have been identified in several species (Mayes & Watson, 2004). Moreover, fatty acid biosynthesis is primarily regulated at the genomic level (Kim *et al*., 1994). Since anabolic implants are composed of steroidal hormones, they could have direct effects on genomic expression of ACC and FAS and may partially explain why implanted animals are

generally leaner than controls.

Decreases in subcutaneous ACC and FAS mRNA concentrations on d 97 of implanted steers compared with non-implanted control steers demonstrated an initial reaction of lipogenic genes to hormonal implants. However, these changes were not noted at later time points in the study. The lack of an effect later in the feeding phase correlates well with our carcass data indicating that type of implant program had no effect on MS, KPH or adjusted fat thickness. However, recent data by Bruns *et al.* (2005) suggested that intramuscular adipose deposition is sensitive to E+TBA implants administered early in the growing period. These authors reported implanting early decreased intramuscular fat content in the LM and decreased MS compared with delayed implanting. Differences in carcass characteristics between our study and Bruns *et al*. (2005) may be related to the amount of time on feed. Steers in our study were on feed for 259 d *vs.* 140 d for cattle fed by Bruns *et al*. (2005). Overall, it is theorized that implanted cattle in our study deposited more muscle and reached physiological maturity later than control steers. However, steers were allowed enough time on feed to deposit sufficient adipose deposition prior to harvest to allow for adequate MS.

Expression of ACC was increased for RPP on d 182 compared to RSP and increased for the average of RSP and RPP on d 259. This is surprising since trenbolone acetate was hypothesized to decrease lipogenic gene expression. Reasons for these findings are currently not known.

Conclusion

Implants increased live animal performance by increasing DMI, ADG and final BW. Carcass characteristics were positively influenced by increasing HCW and enlarging LM area. No differences were noted for serum metabolites. Initial combination implants decreased ACC and FAS gene expression, whereas additional TBA implants increased ACC expression. Albeit a limited number of steers were used, our results suggest that potential decreases in quality grade as a result of implanting cattle are not related to altered lipogenic gene expression in long-fed Holstein steers. Differences in quality grade were noted, which agrees with other studies, and, along with antidotal industry data, it appears that these changes are due to a change in lipogenic gene expression early in the feeding period.

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