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FEEDLOT PERFORMANCE, CARCASS ATTRIBUTES, AND SATELLITE CELL ACTIVITY OF HOLSTEIN STEERS TREATED WITH COMBINATION TRENBOLONE ACETETE AND ESTRADIOL

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FEEDLOT PERFORMANCE, CARCASS ATTRIBUTES, AND SATELLITE CELL ACTIVITY OF HOLSTEIN STEERS TREATED WITH COMBINATION TRENBOLONE ACETETE AND ESTRADIOL IMPLANTS

By

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Abstract

EFFECT OF COMBINATION TRENBOLONE ACETATE AND ESTRADIOL IMPLANTS ON BOVINE SATELLITE CELL PROLIFERATION AND DIFFERENTIATION

By

Jason M. Scheffler

The objective of experiment 1 was to determine the effect of repeated use of growth promoting implants on feedlot performance and carcass characteristics of Holstein cattle. Holstein steers (n = 128) were randomly assigned to one of four treatments that included 0 to 3 implants at 112 d intervals. Component™ TE-S implants were used for all treatments. Repeated administration of Component™ TE-S implants improved average daily gain and resulted in heavier carcasses with larger *longissimus* muscle area ($P < 0.05$). Administration of three successive implants reduced tenderness of Holstein beef, and resulted in advanced skeletal maturity scores ($P<0.05$). The objectives of experiment 2 were to quantify the populations of proliferating and differentiating muscle satellite cells from implanted and control cattle at different body weights. Satellite cells were isolated from the right *semitendinosus* muscle of Holstein steers (n = 24) either implanted 14 d prior to harvest or not implanted and weighing an average of either 220, 390 or 510 kg. The populations of satellite cells, proliferating cells and differentiating satellite cells were identified by immunostain for neural cell adhesion molecule, proliferating cell nuclear antigen and myogenin, respectively. The proportions of proliferating and differentiating satellite cells remained relatively constant in growing cattle from 200 to 500 kg body weight and implants have little effect on satellite cell activity 14 d after implantation ($P>0.05$).

This thesis is dedicated to my parents, Dan and Sue Scheffler.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ADG	Average daily gain
AR	Androgen receptor
BSC	Bovine satellite cell
BW	Body weight
CME	Crushed muscle extract
DES	Diethylstilbesterol
DHT	Dihydrotestosterone
DMEM	Dulbecco's Modified Eagle's Medium
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
E_2	Estradiol
ERα	Estrogen receptor-alpha
FBS	Fetal Bovine Serum
FITC	Fluorescein isothiocyanate
FGF	Fibroblast growth factor
GH	Growth hormone
HCW	Hot carcass weight
HS	Horse serum
IGF-I	Insulin-like growth factor-I
IGFBP	Insulin-like growth factor binding protein
KPH	Kidney pelvic and heart fat

LMA	*Longissimus* muscle area
mRNA	messenger ribonucleic acid
PBS	Phosphate-buffered saline
TBA	Trenbolone acetate
TBOH	Trenbolone
TRITC	Tetramethylrhodamine isothiocyanate
ST	Semitendinosus
SP	Splenius
TB	Triceps brachii
WBS	Warner-Bratzler shear force

Introduction

Since the mid 1940's, producers have used anabolic steroids to improve the rate and efficiency of cattle growth. Starting with the approval (and later banning) of diethylstilbesterol (DES), several estrogenic implants have been approved that contain estradiol-17β, estradiol benzoate or zeranol. Testosterone propionate and trenbolone acetate (TBA) are the only androgenic implants available. Trenbolone acetate, which was approved by the Food and Drug Administration in 1987, is the most recently approved growth promoting implant. Several research trials have focused on the effects of estrogenic and androgenic implants on growth and carcass traits (reviewed by Mader, 1998; Duckett and Andrae, 2001). However, research on the mode of action lags far behind the production aspects of these implants.

Research on the mode of steroid implant action has largely focused on indirect effects, such as changing serum concentrations of various hormones and growth factors that may subsequently increase muscle hypertrophy. Direct effects of estrogens and androgens on muscle hypertrophy are possible because androgen and estrogen receptors are present in skeletal muscle. An increase in satellite cell activity has also been implicated as a contributor to steroid-induced muscle growth. Satellite cells are found between the basal lamina and the plasma membrane of muscle fibers (Mauro, 1961) and are characterized as having scant cytoplasm, dense heterochromatin in the nucleus, and cell shapes that vary from elongated to fusiform to branched (reviewed by Allbrook, 1981; Campion, 1984). Interestingly, satellite cells share no cytoplasm and have no continuity with the myofiber which they are associated with (reviewed by Campion,

1984). Nonetheless, these cells provide the myonuclei necessary for muscle growth and repair. Satellite cells proliferate, differentiate and fuse to adjacent muscle fibers when induced by the appropriate stimulation. Subsequently, the donated nuclei can then become involved in directing muscle protein synthesis (Allen et al., 1983). Thus, the effect of implants on satellite cell activity is potentially a significant factor in defining the effect of implants on muscle growth.

An understanding of cellular events that occur in response to implants may help determine the mechanisms that bring about the improved growth and efficiency that has been highly characterized. Furthermore, understanding the regulation of satellite cell activity may enlighten us to new methods to improve growth and efficiency of livestock species. This information may also allow us to devise new treatments for human diseases such as muscular dystrophy.

Chapter 1

LITERATURE REVIEW

The Role of Satellite Cells in Skeletal Muscle Growth

Skeletal muscles consist of a variable number of elongated, multinucleated fibers. It is generally accepted that the number of fibers does not change significantly after birth (Enesco and Puddy, 1964). Consequently, postnatal muscle growth must occur as the result of muscle fiber hypertrophy. In the *longissimus* muscles of growing hogs, total muscle protein parallels an increase in muscle mass, and age has little effect on the percentage of muscle protein (Harbison et al., 1976). Furthermore, an increase in muscle mass is also highly correlated to an increase in myonuclei. Powell and Aberle (1975) demonstrated that the protein to DNA ratio (pro/DNA) is not different between heavy muscled and light muscled pigs. These data are further supported by research of Harbison et al. (1976), who demonstrated that total DNA in separable muscle was significantly related to separable muscle mass. In addition, total muscle DNA was higher in a muscular line of pigs when compared to an obese line of pigs. Likewise, accumulation of myofiber nuclei in transverse sections is highly correlated to radial myofiber growth in pigs (Swatland, 1977). Collectively, these data show that muscle hypertrophy is the culmination of muscle DNA accretion and subsequent protein accumulation.

Early studies on muscle growth indicated that myonuclei do not divide mitotically and do not synthesize DNA (Stockdale and Holtzer, 1961). Thus DNA must come from a source outside the myofibers. Alexander Mauro first described satellite cells in 1961.

He described these as cells between the plasma membrane of the muscle fiber and the basement membrane. Mauro (1961) hypothesized that satellite cells may serve to regenerate muscle fibers when the muscle tissue is damaged.

Nearly a decade after Mauro's discovery, Moss and Leblond (1970; 1971) demonstrated that satellite cells are indeed the source of myonuclei. Young, male Sherman rats were given an injection of ^{3}H-thymidine and serial slaughtered at intervals from 1 to 72 hrs. The tibialis anterior muscle was removed, fixed, embedded in Epon and sectioned followed by radiographic analysis utilizing an electron microscope. No labeled myonuclei were observed from 1 to 10 hrs although a large number of satellite cells were labeled. This confirmed that myonuclei do not synthesize DNA. However, at 18 hrs a small number of labeled myonuclei were observed. As time progressed, the number of labeled nuclei that were myonuclei increased, thus solidifying the role of satellite cells as the source of myonuclei (Moss and Leblond, 1970, 1971). These data are supported by the findings of Bischoff (1986a) who demonstrated that in the absence of satellite cells, incubation of myofibers in ^{3}H-thymidine produced no labeled myonuclei.

The discovery that satellite cells could be enzymatically released from skeletal muscle tissue opened the door to new research utilizing cell culture (Bischoff, 1974). Numerous studies have looked at the various mechanisms involved in satellite cell proliferation and differentiation *in vitro* (reviewed by Florini, 1987; Dodson et al., 1996; McFarland, 1999). Bischoff (1986b) reported satellite cell activity must be under positive control because death of the associated myofiber had little effect on satellite cell activity, yet mitogens extracted from crushed muscle did induce proliferation of satellite cells. However, Bischoff later found that satellite cells in contact with live myofibers

were less responsive to mitogens than satellite cells liberated from the muscle (Bischoff, 1990). A substantial increase in mitogen stimulated proliferation of satellite cells was observed with marcain killed myofibers compared to cells associated with live myofibers. Together, these data indicate that the mechanisms that control satellite cell activity are complex and may involve several positive and negative stimuli.

Satellite cells behave much like embryonic myoblasts (reviewed by Dayton and Hathaway, 1989). Both cell types proliferate, differentiate and fuse to form myotubes that synthesize muscle specific proteins. Additionally, the response to several growth factors is the same for both satellite cells and embryonic myoblasts. However, there are differences between the two cell types suggest that satellite cells are not just myoblasts that did not fuse during development. For example, myotubes formed by embryonic myoblasts produce three-times more protein than myotubes formed by satellite cells (reviewed by Dayton and Hathaway, 1989). Furthermore, satellite cells possess acetylcholine receptors, which undifferentiated embryonic myoblasts lack.

Postnatal growth reflects differences in satellite cell activity as the animal ages. Di Marco et al. (1987) demonstrated that growth of cattle occurs in three phases. The first phase is a period during which the pro/DNA increases at a faster rate than muscle DNA, indicating protein content is increasing at a disproportional rate versus DNA. In rats, satellite cells consist of about 33% of total muscle nuclei at birth and that percentage drops to a maintenance level of a few percent after 2 months (reviewed by Allbrook, 1981). Furthermore, the absolute number of satellite cells in rat *soleus* muscle increases from 1 to 12 months of age and then decreases to 1 month levels by 24 months (Gibson and Schultz, 1983). These data, taken together, indicate early growth is characterized by

rapid satellite cell proliferation with a large number of satellite cells fusing to myotubes. Thus, the percentage of satellite cells decreases and the percentage of myonuclei involved in protein synthesis increases.

In the second phase, pro/DNA is no longer increasing as rapidly as in phase 1, yet DNA is still increasing (Di Marco et al., 1987). The pro/DNA ratio is not increasing as rapidly, either from less protein production or from increased DNA synthesis. Harbison et al.(1976) demonstrated that the percentage of protein does not change with age, therefore the latter scenario is more likely. An explanation for this change may be that cells are not differentiating and fusing as rapidly, allowing for proliferation of a larger population of satellite cells. The final phase of growth is a period where pro/DNA is no longer increasing and growth is largely attributed to an increase in DNA levels (Di Marco et al., 1987). At this point the growth rate of the animal is starting to slow down. To maintain the pro/DNA at a constant level, after each satellite cell division one daughter cell may fuse and the other will either divide again or become quiescent.

Interestingly, two distinct compartments of satellite cells exist in growing skeletal muscle. Approximately 80% of the satellite cells in a 30 d old rat exhibit a 32 hr cell cycle and likely contribute primarily to muscle growth. The remaining cells take much longer to cycle and may serve as a reserve population to allow for muscle repair (Schultz, 1996). This reserve population is what likely will constitute the maintenance level in mature muscle. Satellite cells in mature muscle are mitotically quiescent as indicated by absence of ^{3}H-thymidine labeled nuclei and the observation that the satellite cells do not have well developed organelles (Schultz et al., 1978). These cells remain quiescent until acted upon by a stimulus such as muscle injury. *In vitro* studies have shown that

exposure of satellite cells to crushed muscle extract (CME) resulted in entrance of satellite cells from mature muscle into DNA synthesis (Bischoff, 1986b). Furthermore, entry into DNA synthesis occurred after a lag of 18 hrs, suggesting that satellite cells in adult muscle are in G_0.

The rate of satellite cell proliferation and differentiation determine the increase in myonuclei during normal skeletal muscle growth (Moss and Leblond, 1971; Campion, 1984). Consequently, it is logical to think that modulating the relative proliferation and differentiation rates of satellite cells may allow us to exert control over the rate of muscle growth. An understanding of the mechanisms involved in satellite cell proliferation, differentiation, and fusion will be a powerful tool in the optimization of meat production efficiency.

Three theories about the regulation of the transition from proliferating myoblast to non-proliferating myotube have been postulated (reviewed by Dayton and Hathaway, 1989). The first theory is that cells go through "quantal mitosis". This theory states that with each cell division, the daughter cells have taken a step toward terminal differentiation. Each step is a small change in gene expression. After a series of divisions, perhaps a fixed number, cells will take the final step to terminally differentiate and irreversibly withdraw from the cell cycle when they can then fuse to existing myotubes. This theory calls into question what changes in gene expression need to take place in that final division for a cell to both withdraw from the cell cycle and become fusion-competent. A second theory states that as a cell goes through several divisions, the time spent in G_1 increases thus increasing the probability that the cell will fuse to an existing myotube. Therefore, it is the process of fusion that causes the cell to withdraw

from the cell cycle. This theory has been proven inaccurate by data demonstrating that satellite cells can withdraw from the cell cycle and commit to differentiation prior to fusion (Nadal-Ginard, 1978). The final and most widely accepted theory states that at the beginning of G_1, myoblasts either continue through the cell cycle or withdraw from the cell cycle, differentiate and fuse based on presence or absence of various mitogenic factors. This theory agrees with the widely held concept that a reduction in serum concentration, or a reduction in the concentration of various growth factors can induce differentiation of satellite cells *in vitro* (McFarland et al., 1988; Dodson et al., 1990; Greene and Allen, 1991; Brameld et al., 1998; McFarland, 1999).

Regulation of satellite cell activity is more complex. For example, Fibroblast growth factor (FGF) can inhibit differentiation without inducing proliferation. Under certain conditions, FGF may induce progression of the cell out of G_0 and stop it at a new restriction point in G_1, thus preventing the first step towards differentiation (reviewed by Florini, 1989). However, this new restriction point has not been defined in biochemical terms. Coolican et al. (1997) demonstrated that insulin-like growth factor-I (IGF-I) can stimulate both proliferation and differentiation through two separate intracellular signaling pathways. Finally, the removal of growth factors by serum deprivation can trigger the cells to irreversibly enter into apoptosis (Mampuru et al., 1996). Consequently, it appears that satellite cell regulation is a very dynamic process. Satellite cells respond to the spectrum of chemical signals in their environment by speeding up, slowing down or turning on or off certain biochemical pathways. The coordination of all these changes determines if the cell will proliferate, differentiate, become quiescent, or undergo apoptosis.

Effects of Anabolic Steroids on Skeletal Muscle Growth

Implanting steers with combination TBA and estradiol (E_2) implants increases the serum concentration of trenbolone (TBOH) and E_2 (Lee et al., 1990; Hayden et al., 1992) . Once in the serum, these agents can act on several potential target organs including reproductive tissues, endocrine glands, the liver and skeletal muscle. These hormones have several indirect and potential direct effects on skeletal muscle hypertrophy. Thompson et al. (1989) demonstrated that serum from Sprague-Dawley rats treated with TBOH increases rat satellite cell proliferation *in vitro* compared to serum from non-treated rats. Similar results were observed in satellite cells isolated from steers treated with combination TBA/E_2 implants (Johnson et al., 1998a). Collectively, these data indicate that anabolic implants elicit a response from satellite cells that may contribute to the increased muscle mass observed in treated animals.

Growth hormone (GH) is frequently measured to monitor the effect of anabolic steroids. Gopinath and Kitts (1984) found an elevated secretion rate of GH in steers treated with estrogenic agents (DES, zeranol or estradiol benzoate and progesterone). In addition, GH secretion rate was highly correlated with growth rate over the course of the study (Gopinath and Kitts, 1984). Breier et al. (1988a) found that estradiol-17β implants increase GH release independent of nutritional status. Grigsby and Trenkle (1986) showed E_2 implantation resulted in more frequent GH secretory peaks in Simmental, Limousin and Angus cattle but did not influence the amplitude of the secretory peaks. Cattle implanted with androgenic implants have also shown increases in serum GH concentrations and muscle growth (Hunt et al., 1991a; Moran et al., 1991).

Growth hormone receptors on skeletal muscle have been identified in the human (Marcell et al., 2001) and in the pig (Louveau and Etherton, 1992). Furthermore, GH has been shown to increase chicken satellite cell proliferation and decrease differentiation (Halevy et al., 1996; Hodik et al., 1997). However, there has not been strong support for a direct effect of GH on mammalian satellite cell activity or protein accretion. Allen et al. (1983) showed that an increase in serum from 3 to 15% increases porcine myofiber α-actin production *in vitro* in a dose dependent manner. However, titration of GH in the media had no effect on protein accretion.

The role of GH in skeletal muscle hypertrophy may be attributed to the stimulation of IGF-I release from the liver. Growth hormone receptors have been found on liver membranes and hepatocytes (Boyd and Bauman, 1989) and Breier et al. (1988b) demonstrated increased circulating GH may stimulate an increase in GH receptors in the liver and result in increased IGF-I production. Johnson et al. (1998b) showed that lambs implanted with TBA/E_2 had 150% higher steady-state hepatic IGF-I mRNA levels than control lambs. Interestingly, steers receiving either a TBA implant (140mg), a TBA/E_2 implant (120mg/24mg) or a E_2/progesterone implant (20mg/200mg) had larger livers than steers not receiving an implant (Hutcheson et al., 1997). This may potentially contribute to increased IGF-I production. However, the liver is not the only source for IGF-I. Roith et al. (2001) reviewed several articles indicating that IGF-I can be produced locally in many tissues including skeletal muscle. Furthermore, locally produced IGF-I may have a more profound effect on muscle growth than circulating IGF-I (Le Roith et al., 2001). In fact, TBA/E_2 implants can increase IGF-I mRNA levels in *longissimus* muscle from TBA/E_2 implanted sheep (Johnson et al., 1998b).

The role of IGF-I in muscle growth has been characterized in several studies. Muscle hypertrophy increased at one month of age in mice with IGF-I overexpression. However, this increase was diminished with age (Chakravarthy et al., 2001). In cattle, plasma IGF-I levels increased progressively with age in both bulls and steers (Lee et al., 1990). It is generally accepted that bulls are heavier muscled than steers, and this corresponds with a larger increase in IGF-I over time in bulls and subsequent higher overall IGF-I concentrations (Lee et al., 1990). However, a combination TBA/E_2 implant in steers eliminated the difference between steers and bulls.

Insulin-like growth factor-I can induce proliferation and differentiation of satellite cells in culture (reviewed by Florini, 1987). Satellite cells harvested after one month of IGF-I overexpression in striated muscle produced larger colonies than cells from wild-type mice (Chakravarthy et al., 2001). Interestingly, this increased growth rate was not maintained in cells harvested at 18 months of age. Addition of long R3-IGF-I (a more biologically active IGF-I with lower affinity to IGF binding proteins) could not further stimulate proliferation in cells from 18-month-old transgenic mice.

While increased muscle growth may result from the action of anabolic agents, growth can also increase by the reduction of catabolic agents such as glucocorticoids. In Wistar albino rats receiving glucocorticoid injections, protein synthesis rate was decreased in a similar manner to diabetic, hypophysectomized or starved rats (Millward et al., 1976). In livestock, Simmental cattle tend to have lower plasma glucocorticoids than Angus cattle supporting the conclusion that faster growing cattle have lower plasma glucocorticoid levels (Grigsby and Trenkle, 1986). Furthermore, serum cortisol levels are higher in steers than bulls, likely contributing to the reduced gain and increased rib fat

content in steers (Lee et al., 1990). Thus, a potential role for anabolic implants may be to reduce serum glucocorticoid levels.

Jones et al. (1991) showed that TBA reduced cortisol levels in bulls during the growing phase. Additionally, TBA/E_2 implants tended to reduce the serum cortisol concentrations in steers during the growing phase, and significantly reduced serum cortisol during the finishing phase when compared to non-implanted controls (Lee et al., 1990). Testosterone, TBA and dihydrotestosterone (DHT) decreased cortisol synthesis in both adrenocorticotrophic hormone (ACTH) and non-ACTH stimulated adrenocortical cells in culture (Isaacson et al., 1993). It is of note that E_2 implants have little effect on plasma glucocorticoid concentrations (Grigsby and Trenkle, 1986; Hayden et al., 1992). However, Isaacson and Jones (1993) found that zeranol decreased cortisol production in adrenocortical cells, but only when those cells were stimulated with ACTH.

Although it appears that androgenic and estrogenic implants act largely through indirect action on muscle and adipose tissue, there is evidence that they may also act through direct mechanisms. Treatment of steers with anabolic agents results in larger muscles in the neck and shoulder regions giving a more bull-like muscle distribution (Wood et al., 1986; Foutz et al., 1997). Muscles such as the *levator ani* involved in sexual reproduction have been shown to regress due to castration, while testosterone propionate replacement therapy successfully restores muscle weight to intact male levels (Boissonneault, 2001). Castration of Holtzman/Sprague-Dawley rats resulted in a reduction in weight in the *bulbocavernosus* and *levator ani* muscles with no effect on the *plantaris* muscle. Subsequent treatment with DHT resulted in growth comparable to non-

castrated rats in the *bulbocavernosus* and *levator ani* muscles, however no effect on the *plantaris* muscle was observed (Antonio et al., 1999)

Androgen receptors (AR) have been found in skeletal muscle and the relative levels of AR differ between muscles (Sauerwein and Meyer, 1989). Antonio et al. (1999) showed AR levels in the *bulbocavernous* and *levator ani* muscles of Holtzman/Sprague-Dawley rats were three times higher than those found in the *plantaris* muscle. The differences in AR levels can be explained by their respective roles in sexual reproduction. The *bulbocavernosus* and *levator ani* muscles are involved in copulation and ejaculation (Blanco et al., 1995) and decrease in weight after castration (Antonio et al., 1999). Conversely, the *plantaris* muscle is not involved in sexual reproduction and accordingly, is not affected by castration nor is it sensitive to DHT treatment (Antonio et al., 1999). In bulls, AR mRNA levels are relatively high in the *triceps brachii* (TB) and *semitendinosus* (ST) muscles and low in the *splenius* (SP) at 4 months of age. However by 16 months, AR mRNA levels increase in the SP to be comparable to ST AR mRNA levels but still less than levels found in the TB (Brandsetter et al., 2000). The AR differences observed in these three muscles may contribute to the allometric growth of these muscles. The ST and TB both grow at high rates early in life while the SP is a sexually dimorphic muscle that will likely have the most significant growth later in life (Brandsetter et al., 2000). The increase in SP AR levels may be correlated to an increase in serum testosterone levels during the growth phase as was observed in bulls (Lee et al., 1990).

Testosterone was found to increase the amount of AR in actively growing satellite cells *in vitro* (Doumit et al., 1996). Likewise, castration significantly reduced AR levels

in the *bulbocavernous* and *levator ani* muscles, but subsequent treatment with *dihydrotestosterone* resulted in AR levels comparable to non-castrated rats (Antonio et al., 1999). Antonio et al. (1999) postulated that increased AR levels due to administration of pharmacological levels of androgens may enhance the responsiveness of muscles that normally have minor or no response to androgens. Castration of bulls resulted in a trend for increased AR mRNA in TB and ST muscles from 4 to 16 months. Thus, testicular steroids may negatively regulate AR expression (Brandsetter et al., 2000). Interestingly, injection of testosterone (1 mg/kg of body mass) into white male rats caused a rapid decrease in AR binding in skeletal muscle cytosol from time zero to 60 min. Binding then increased to 200% of the control level 6 hr after injection and a high level of binding was maintained until 18 hr before regressing back to control levels by 28 hr (Osipova-Goldberg et al., 2001). Certainly there are considerations that need to be taken into account when comparing across experiments. For example, mRNA levels do not necessarily translate into comparable protein levels. The time of sampling, muscles and species involved may influence the interpretation of the data. Nonetheless, to summarize these data, AR abundance in sexually dimorphic muscles varies more than levels in skeletal muscle in general. The concentration of androgens in the muscle affects androgen receptor levels.

Estrogen receptors have been found in bovine skeletal muscle (Meyer and Rapp, 1985; Sauerwein and Meyer, 1989), albeit in lower concentrations than found in reproductive tissue. Thus estradiol may have direct effects on skeletal muscle growth. Furthermore, E_2 can compete with androgens to bind the AR, potentially eliciting an

antagonistic effect (Sauerwein and Meyer, 1989). The changes in gene expression due to the estrogen binding a receptor in skeletal muscle have not been characterized.

Recent research using a yeast and mammalian two-hybrid system has shown that the AR and estrogen receptor-α (ERα) interact directly (Panet-Raymond et al., 2000). In a mammalian two-hybrid system, ERα complex can modulate the activity of AR as demonstrated using a reporter gene (MMTV-GH) containing an androgen response region and the human GH gene. In the presence of both E_2 and a synthetic testosterone analog, mibolerone, a 35% reduction in MMTV-GH activity compared to mibolerone alone in CV-1 cells cotransfected with both AR and ERα cDNAs was observed (Panet-Raymond et al., 2000). Thus, there is a steroid dependent interaction between AR and ERα. An interaction between AR and ERα resulting in decreased GH production does not fit with the additive effects on muscle growth observed in cattle implanted with combination implants (Herschler et al., 1995). This interaction may constitute a rather minor pathway overcome by the total metabolic changes in cattle as the result of treatment with an anabolic implant, or there may be no significant effect. Alternatively, target cells may have a prevalence of one receptor or the other thus limiting this interaction.

Although skeletal muscle tissue and satellite cells may possess receptors for androgens, those receptors may not function to induce satellite cell proliferation or differentiation or cause muscle hypertrophy. Allen et al. (1983) showed addition of GH or testosterone to growth media containing 10% porcine serum had no effect on fusion percentage or muscle fiber α-actin accretion in satellite cells isolated from neonatal, rapid growing or adult rats indicating that the direct effect of testosterone on satellite cells may

be minimal. Furthermore, direct addition of TBA to rat satellite cell cultures had no effect on satellite cell proliferation or myotube nuclei accretion, nor did it have any synergistic effect with IGF-I or FGF on proliferation or differentiation (Thompson et al., 1989). Powers and Florini (1975) demonstrated testosterone increases the thymidine labeling index of rat satellite cells to a modest degree. Support of these data is limited to the studies that demonstrate the presence of a cytoplasmic androgen receptor as studies that demonstrating a direct effect of testosterone on skeletal muscle have not been forthcoming (reviewed by Florini, 1987). More recently, Doumit et al. (1996) demonstrated that satellite cells posses nuclear androgen receptors and that these receptor levels are unregulated by testosterone administration. Additionally, satellite cell differentiation was reduced in response to testosterone, but proliferation was unaffected.

Certainly, the mechanisms involved in the regulation of satellite activity are complex and ongoing research may actually add to this complexity. The potential direct effects of GH and testosterone are still being debated. Additionally, research in the past 20 years showing skeletal muscle can produce IGF-I locally has changed the perception of the somatotrophic axis which may be further complicated if a direct effect of GH on skeletal muscle can be demonstrated. Deciphering the mode of action of implant growth promoters is further complicated by the fact that both TBA and E_2 may have direct and indirect effects on a multitude of tissues. Yet understanding what is happening at the cellular level and devising methods that allow us to measure biochemical changes *in vivo* may allow us to understand the mode of action of anabolic implants.

Chapter 2

EFFECT OF REPEATED ADMINISTRATION OF COMBINATION TRENBOLONE ACETATE AND ESTRADIOL IMPLANTS ON GROWTH, CARCASS TRAITS AND BEEF QUALITY OF LONG-FED HOLSTEIN STEERS

Abstract

The objective was to determine the effect of repeated use of implants on feedlot performance and carcass characteristics of Holstein cattle. Holstein steers (n = 128) weighing an average of 211 kg were blocked by weight and randomly assigned to 16 pens. At the initiation of the trial (d 0), pens were assigned to one of four treatments: non-implanted control (C), implant on d 0, d 112 and d 224 (T3), implant on d 112 and d 224 (T2) and implant on d 224 (T1). Component™ TE-S implants (120 mg trenbolone acetate and 24 mg estradiol per implant) were used for all treatments during the 291 d feeding period. Within each time period, implanted steers had higher average daily gain (ADG) compared to non-implanted steers ($P < 0.05$). Steers were harvested at a commercial abattoir on d 291. Hot carcass weights of T2 and T3 were similar to each other and greater than C and T1 ($P < 0.05$). Dressing percentage, adjusted 12^{th} rib fat, percentage kidney pelvic and heart fat, yield grade and CIE L*a*b* color values were not different among treatments. *Longissimus* muscle areas (LMA) of T2 and T3 carcasses were larger than LMA of C ($P < 0.01$). No USDA Select carcasses were produced from control cattle, while the percentage of Select from implanted cattle ranged from 10 to 18%. Skeletal maturity advanced progressively with each additional implant. Steaks from T3 carcasses had a higher percentage of protein than controls ($P < 0.05$) and were

less tender than all other treatments ($P < 0.05$). Repeated administration of combination trenbolone acetate and estradiol implants improved ADG and resulted in heavier carcasses with larger LMA. Administration of three successive implants reduced tenderness of Holstein beef, and resulted in advanced bone maturity scores.
Keywords: Implant, Holstein, tenderness

Introduction

The Food and Drug Administration approved trenbolone acetate in 1987 for use in implants to increase rate of gain and feed efficiency of cattle. The use of growth promoting implants is currently widespread. In 1999, over 96% of all cattle in feedlots were implanted at least once (NAHMS, 2000). Combination trenbolone acetate and estradiol (TBA/E_2) implants improve average daily gain (ADG) and feed efficiency by 6 to 15% and 4 to 13%, respectively, compared to non-implanted cattle (Johnson et al., 1996; Foutz et al., 1997; Hermesmeyer et al., 2000). However, combination implants may have deleterious effects on USDA Quality Grade (Herschler et al., 1995; Foutz et al., 1997) and tenderness of beef (Thonney et al., 1991; Roeber et al., 2000). In contrast, several studies have shown no effect of combination implants on quality grade (Hunt et al., 1991b; Gerken et al., 1995; Johnson et al., 1996), or *longissimus* muscle tenderness (Apple et al., 1991; Hunt et al., 1991b; Gerken et al., 1995).

Repeated implanting of cattle with anabolic agents is common. This is particularly true for Holstein steers that are fed for longer time periods and to heavier weights than cattle of beef breeds. However, it is not clear if growth benefits or compromised quality result from repeated use of implants. Another question of interest is

whether implants administered early in life decrease the effectiveness of implants given later. While several studies have evaluated the effect of various implants and implant strategies on beef type steers, few studies have examined the effect of repeated use of TBA/E_2 implants on dairy type steers. Therefore, the objectives of this study were to determine the effect of implant strategy on animal growth, carcass characteristics, and meat quality of Holstein steers fed a high concentrate diet for 290 days.

Materials and Methods

Holstein steers (n = 170; 180 kg) were purchased and transported to the Michigan State University (MSU) Beef Cattle Teaching and Research Center and given a 56 d adjustment period. After the adjustment period, 128 uniform steers were selected, blocked by weight and assigned to 16 pens, each containing eight steers. At the initiation of the trial (d 0), pens were assigned to one of four treatments: non-implanted control (C), implant on d 0, d 112 and d 224 (T3), implant on d 112 and d 224 (T2) and implant on d 224 (T1). Component™ TE-S implants (120 mg TBA and 24 mg E_2 per implant; Vet-Life, West Des Moines, IA), administered subcutaneously to the posterior aspect of the ear using an implant gun, were used for all treatments during the 291 d feeding period. Initial and final weights were compiled from the average of weights taken on two consecutive days. Steers were weighed every 28 d throughout the trial. Steers were fed ad libitum a diet consisting of 72.9% corn, 16.9% corn silage and 10.2% protein and mineral mixture (Table 1) once daily and feed disappearance was calculated on a pen basis. All procedures were approved by the MSU committee on Animal Use and Care (approval # 04/99-051-00).

Table 1. Composition of diet[a]

	% of diet DM
High moisture corn	72.9
Corn Silage	16.9
Soybean meal	7.6
Calcium carbonate	1.105
Trace mineral salt	0.502
Urea	0.375
Potassium chloride	0.280
Dicalcium phosphate	0.111
Ground corn	0.167
Selenium 90	0.053
Vitamin A	0.0080
Rumensin 80	0.0139

[a]Diet was formulated to provide 13% crude protein.

Carcass data collection. On d 291, all steers were transported to a commercial abattoir. Hot carcass weights (HCW) were measured before and after removal of kidney, pelvic and heart fat (KPH). The percentage of KPH was determined by weight difference after removal of KPH. After carcasses were chilled for forty-eight h, two independent evaluators determined *longissimus* muscle area (LMA; cm^2) and 12th rib fat (cm). Yield grade was calculated (USDA, 1997). Marbling score was determined by three independent evaluators using a scale where 300 = slight^0 and 800 = moderately abundant^0. Skeletal maturity was determined based on subjective evaluation of ossification of cartilage associated with the sacral, lumbar and thoracic vertebra. One evaluator estimated skeletal maturity using a scale where 0 = A^0 and 100 = B^0. USDA Quality Grade determined by a USDA beef carcass grader was also recorded.

A rib section adjacent to the 11^{th} and 12^{th} ribs was removed from the right side of each carcass and transported to the MSU Meat Laboratory. A 1 cm slice of *longissimus* muscle adjacent to the 12^{th} rib was trimmed, diced and frozen at –20°C for subsequent determination of proximate composition. Color (CIE L*a*b*) of the ribeye (allowed to bloom for approximately 15 min) was evaluated using a Minolta chroma meter (Ramsey, NJ). The remaining rib sections were vacuum-packaged, aged for a total of 14 days at 4°C and frozen at –20°C until tenderness analysis by Warner-Bratzler shear force.

Proximate composition. Frozen samples were milled with dry ice and carbon dioxide was allowed to evaporate for two days at 4°C. Moisture content was measured by air-drying (AOAC, 1995; Method 950.46B). Total fat was determined by using a Soxtec Fat Analyzer (AOAC, 1995; Solvent Extraction Method 991.36; Tecator,

Höganäs, Sweden). Crude protein was determined by using combustion method 992.15 (AOAC, 1995; Leco FP-2000, Leco Corp., St Joseph, MI).

Tenderness by Warner-Bratzler shear force. One 2.54 cm thick steak was cut from each frozen rib section and allowed to thaw for 24 h at 4°C. Steaks were cooked on a clamshell grill (model QS24; Taylor Co; Rockton, IL). Temperature of the upper plate was set at 104.4°C and the bottom plate was set at 102.8°C with a 2.16 cm gap between plates. Four to 5 steaks were cooked simultaneously and copper constantan thermocouples (0.051 cm diameter, 15.2 cm length; Omega Engineering Inc.; Stamford, CT) were inserted into one steak per batch to monitor temperature increase during cooking. Post-cook temperature rise was monitored in each steak with small diameter hypodermic probe thermocouples (0.089 cm diameter, 5.72 cm length; Cole-Parmer; Vernon Hills, IL). Steaks were cooked for 450 s, to a final internal temperature of 72°C ± 1.5°C. Steaks were allowed to cool to room temperature and then were chilled at 4°C overnight. Six, 1.27 cm cores were taken parallel to the longitudinal axis of the muscle fibers using a drill press-mounted corer. Cores were sheared perpendicular to the fibers using a Warner-Bratzler head on a TA-HDi Texture Analyzer (Texture Technologies Corp., Scotsdale, NY). The crosshead speed was set at 3.30 mm/s.

Statistical Analysis. During the course of the study three steers were removed from the trial for health reasons. Five steers that exhibited ADG that were 2 standard deviations below the mean of the treatment for two consecutive weigh periods were removed from the study. Records for these steers were excluded from the data set and feed consumption records for their respective pens were adjusted according to net energy requirements for these steers. One control steak was omitted from the tenderness analysis

due to inability to obtain cores without excessive visible connective tissue. Mean shear force for this steak was more than six standard deviations away from the overall mean for the treatment. The data were analyzed using the general linear model procedures of SAS (SAS Inst. Inc., Cary, NC). Pen means for feed consumption were used for computing feed efficiency. Average daily gain, carcass data, and meat characteristics were analyzed with animal as the experimental unit. The model included treatment and pen within treatment as the main effects. Least squares means were used to account for the unequal number of steers in each treatment group. The distributions of quality grades were compared using the chi-square option of the frequency procedure of SAS.

Results and Discussion

Growth and feed efficiency. In each time period, cattle receiving implants had a greater ADG than non-implanted cattle ($P < 0.05$; Table 2). Over the course of the study, T2 and T3 cattle had greater ADG and final weights compared to C and T1 cattle ($P < 0.05$). Cattle receiving second and third implants had ADG similar to cattle receiving their initial implant within the same time period. Overall, T3 cattle consumed more dry matter than C and T1 ($P < 0.05$). Implant treatments T1 and T2 improved overall gain:feed compared to control cattle ($P < 0.05$; Table 2). Cattle receiving their first implant generally exhibited the greatest numerical improvement in feed conversion efficiency. However, cattle in treatment T3 did not have an improved gain:feed after the first or subsequent implants. This may be attributed to administration of the first implant at lighter weights when cattle are relatively more efficient converters of feed into gain, and a diminished response to subsequent implants. Likewise, Mader et al. (1985) and

Table 2. Effect of a combined trenbolone acetate and estradiol implant on feedlot performance of Holstein steers

	Implant treatment[a]				
	C	T1	T2	T3	SEM
No. of Steers	31	30	28	31	
Initial weight, kg	213	213	212	213	1.9
Final weight, kg	587[b]	591[b]	629[c]	645[c]	7.1
Average Daily gain, kg/d					
d 0-d112	1.50[b]	1.41[c]	1.50[b]	1.72[d]	0.03
d 113-224	1.24[b]	1.20[b]	1.49[c]	1.45[c]	0.03
d 225-291	1.04[b]	1.31[c]	1.28[c]	1.20[c]	0.05
d 0-291	1.29[b]	1.30[b]	1.44[c]	1.49[c]	0.03
Carcass adjusted gain, kg/d[c]	1.28[b]	1.31[b]	1.44[c]	1.49[c]	0.02
Number of pens	4	4	4	4	
Dry matter intake, kg/d					
d 0-d112	6.86	6.50	6.79	7.10	0.33
d 113-224	10.06[bc]	9.24[b]	10.20[bc]	11.46[c]	0.33
d 225-291	8.60[bc]	7.71[b]	9.22[c]	9.52[c]	0.38
d 0-291	8.54[b]	7.87[b]	8.63[bc]	9.37[c]	0.25
Gain / feed					
d 0-112	0.221	0.218	0.221	0.245	0.010
d 113-224	0.124[b]	0.143[bc]	0.147[c]	0.127[bc]	0.007
d 225-291	0.121[b]	0.169[d]	0.139[c]	0.127[bc]	0.005
d 0-291	0.152[b]	0.172[c]	0.167[c]	0.160[bc]	0.005

[a]Implant strategy: C = no implant, T1= no implant/ no implant/ implant, T2= no implant/ implant/ implant, T3= implant/ implant/ implant.

[b,c,d]Means with unlike superscripts within a row differ ($P < 0.05$).

[c]Based on mean dressing percentage of 58.1.

Simms et al. (1988) demonstrated that zeranol implants given before the finishing phase tended to decrease gain:feed of reimplanted steers during the finishing phase. Using Holstein steers fed to a small degree of marbling endpoint, Perry et al. (1991) showed implanted cattle had an increase in ADG and dry matter intake (DMI) with a decrease in DMI per body weight (BW) gain. Additionally, TBA/E_2 implants improved ADG and feed efficiency by 6 to15% and 4 to13% respectively, compared to non-implanted cattle (Johnson et al., 1996; Foutz et al., 1997; Hermesmeyer et al., 2000). These studies ranged from 90 to151 d in length and the steers were crossbred beef cattle.

Carcass attributes. Carcasses from T2 and T3 cattle were heavier than carcasses from C and T1 ($P < 0.05$; Table 3). Heavier T2 and T3 carcass weights reflect higher final live weights at harvest, because dressing percentage did not differ among treatments. Increased carcass weights in response to implant treatments have been previously reported (Foutz et al., 1997; Hermesmeyer et al., 2000; Roeber et al., 2000).

Perry et al. (1991) showed that a combination implant (140 mg TBA and 28 mg E_2) did not affect LMA of Holstein steers fed to a small degree of marbling (approximately 216 d). Johnson et al. (1996) reported that a single Revalor-S implant (120 mg TBA and 28 mg E_2) resulted in increased LMA at d 115, but had little effect at 143 d. Conversely, Roeber et al. (2000) showed that implantation with Revalor-S at d 0 and 59 of a 140 d feeding period increased LMA. Foutz et al. (1997) also demonstrated that implants containing TBA resulted in increased LMA. Our data corroborates Foutz et al. (1997) and Roeber et al. (2000) as LMA for T2 and T3 were greater than control LMA, while T3 was also greater than T1 ($P < 0.05$; Table 3). One implant for 67 d (T1) was not sufficient to increase LMA in the current study. This is in general agreement

Table 3. Effect of a combined trenbolone acetate and estradiol implant on carcass and *longissimus muscle* traits of Holstein steers

	Implant treatment[a]				
	C	T1	T2	T3	SEM
No. of steers	31	30	28	31	
Hot carcass weight, kg	340[f]	345[f]	366[g]	375[g]	3.9
Dressing %[b]	57.8	58.1	58.2	58.1	0.27
KPH, %[c]	3.1	3.0	3.2	3.4	0.27
Longissimus muscle area, cm^2	74.7[f]	76.7[fg]	80.4[gh]	83.0[h]	1.38
12th rib fat, mm	7.1	6.7	6.7	7.4	0.45
Yield Grade	3.0	2.9	2.9	2.9	0.09
Longissimus muscle color					
L*	36.8	36.7	36.8	36.0	0.43
a*	24.4	23.9	24.6	24.5	0.37
b*	12.3	11.9	12.3	12.3	0.28
Skeletal maturity[d]	55[f]	61[g]	78[h]	88[i]	1.6
Proximate analysis					
Crude protein, %	21.0[f]	21.2[fg]	21.2[fg]	21.5[g]	0.16
Moisture, %	71.7	72.5	71.9	72.1	0.38
Ether extract, %	5.7	5.0	5.5	5.0	0.45
Marbling score[e]	482[g]	430[f]	451[fg]	446[fg]	18.7

[a]Implant strategy: C = no implant, T1= no implant/ no implant/ implant, T2= no implant/ implant/ implant, T3= implant/ implant/ implant.

[b]Dressing percent is based on the unshrunk live weight.

[c]KPH=Kidney, pelvic and heart fat.

[d]$50 = A^{50}$ and $100 = B^{0}$.

[e]300 = slight[0] and 800 = moderately abundant[0].

[f,g,h,i]Means with unlike superscripts within a row differ ($P < 0.05$).

with the studies of Perry et al. (1991) and Johnson et al. (1996). However, the implant effects on LMA may have been diminished in these studies if cattle were fed beyond the effective payout period of the implant. This is supported by the fact that Johnson observed differences in LMA at d 115, but not at d 143. Although LMA and HCW were increased in T2 and T3 in the current study, the relationship between LMA and HCW were similar among treatments ($P > 0.05$; data not shown).

No differences between treatments were found for dressing percentage, percentage KPH fat, 12^{th} rib fat, or yield grade ($P > 0.05$; Table 3). Perry et al. (1991) and Johnson et al. (1996) found no differences in dressing percentage and 12th rib fat for implanted steers. However, implants have been shown to decrease the percentage of KPH for implanted steers (Johnson et al., 1996; Duckett et al., 1999; Roeber et al., 2000). These differences may be attributed to the manner in which KPH was measured. In this study, KPH was measured by carcass weight difference before and after removal of KPH, whereas the afore mentioned studies used a subjective measure by trained personnel. It is also possible that combination implants do not affect KPH fat in Holstein steers to the same extent as in beef steers.

Implant treatments did not affect CIE L*a*b* color values of the *longissimus* muscle ($P > 0.05$; Table 3). One dark cutter was observed in this study in the T3 treatment group. Herschler et al.(1995) demonstrated that implant treatments resulted in darker *longissimus* muscle, while Scanga et al. (1998) showed combination androgen and estrogen implants result in a higher incidence of dark cutters. Skeletal maturity was advanced by successive implant treatments in the current study ($P > 0.05$). Similarly,

Foutz et al. (1997) and Roeber et al. (2000) observed advanced maturity score of carcasses from steers receiving a single TBA/E_2 implant.

Longissimus muscle crude protein was higher ($P<0.05$) in steers receiving three implants (T3) compared to controls (Table 3). This was accompanied by a numerical increase in moisture and decrease in ether extract, although these traits were not statistically different among treatments. Similarly, Johnson et al. (1996) showed that compared to control steers, longissimus muscle of implanted steers had a higher percentage of moisture, tended to have higher percentage of protein and no change in percentage of fat. Foutz et al. (1997) found no change in percentage of protein, moisture or fat due to a single TBA/E_2 implant.

Marbling scores from T1 carcasses were lower than scores from C carcasses (Table 3). Duckett et al. (1999) showed that implanting with a 200 mg TBA/ 28 mg E_2 implant reduced marbling score by one half degree, while reimplanting did not further reduce marbling score. Roeber et al. (2000) found a decrease in marbling score in steers implanted with Revalor-S on d 0 and 59 of a 140 d feeding period. Conversely, Perry et al. (1991) and Foutz et al. (1997) found no change in marbling score as the result of implant treatments containing 140 mg TBA/28 mg E_2 and 140 mg TBA/20 mg E_2, respectively. Both Duckett et al. (1999) and Roeber et al. (2000) used more aggressive implant strategies, reimplanting after a much shorter feeding period than Perry et al. (1991), Foutz et al. (1997) or what was used in the current study. These data, taken together indicate that there is a threshold of TBA and E_2 that can be utilized before a significant loss of marbling will be noted. The distribution of quality grades as determined by a USDA employee was not different across treatments ($P > 0.05$; Table 4).

A numerical increase in percentage of USDA Select carcasses was found for the implant treatments. Roeber et al. (2000) showed a 30% decrease in carcasses grading USDA Prime or Choice for implanted versus non-implanted beef cattle. Differences between this and the current study may be attributed to the aggressive implant strategy used and the use of beef type breeds by Roeber et al. (2000).

Warner-Bratzler shear force. The average ribeye steak tenderness, measured by Warner-Bratzler shear force (WBS), was acceptable for all treatments. However, ribeye steaks from T3 cattle had higher shear force values than steaks from other treatments (Table 5). Additionally, 2 steaks from T3 had inferior shear values (WBS > 5 kg), whereas all remaining steaks were of acceptable tenderness (WBS $\leq$ 4.5 kg). Roeber et al. (2000) found that steaks from cattle receiving repeated TBA/E_2 implants were as tender as steaks from control steers, whereas steaks from cattle receiving a single TBA/E_2 implant were tougher than control steaks. In addition, Foutz et al. (1997) found implant treatments significantly increased *longissimus* muscle shear force.

Conclusions

Results of this study showed average daily gain for the control group numerically declined in each time period. Implants appeared to attenuate this decline by stimulating the growth rate. Repeated implant strategies increase ADG, HCW and LMA, without affecting dressing percent, adjusted 12th rib fat, percentage of KPH, yield grade or color. Marbling scores and the proportion of USDA Select carcasses from cattle receiving repeated implants were not statistically different from non-implanted cattle in this study. However, the control treatment produced no carcasses grading Select, whereas 10 to18%

Table 4. USDA Quality Grade distribution from Holstein steers treated with a combination trenbolone acetate and estradiol implant[ab]

	Implant treatment[c]							
	C		T1		T2		T3[d]	
	n	%	n	%	n	%	n	%
USDA Prime	5	16.1	2	6.7	7	25.0	3	10.0
USDA Choice	26	83.9	25	83.3	16	57.1	24	80.0
USDA Select	0	0.0	3	10.0	5	17.9	3	10.0
Total	31		30		28		30	

[a]Quality grade determined by USDA employee.

[b]Distribution of quality grades across treatments was not different (P = 0.09).

[c]Implant strategy: C = no implant, T1= no implant/ no implant/ implant, T2= no implant/ implant/ implant, T3= implant/ implant/ implant.

[d]One dark cutting carcass was not graded.

Table 5. Effect of combination trenbolone acetate and estradiol implants on cooking properties of rib steaks from Holstein Steers

	Implant treatment[a]				
	C	T1	T2	T3	SEM
Number of steaks	30	30	28	31	
Cooking loss, %	15.1	15.2	15.4	15.1	0.62
Cooked steak weight, g	249	257	257	263	6.3
Shear force, kg	2.5[b]	2.6[b]	2.8[b]	3.1[c]	0.11

[a]Implant strategy: C = no implant, T1= no implant/ no implant/ implant, T2= no implant/ implant/ implant, T3= implant/ implant/ implant.

[b,c]Means with unlike superscripts within a row differ (P<.05).

of carcasses from implanted cattle graded USDA Select. Multiple implants can result in less tender steaks and advanced skeletal maturity. The most effective implant strategy in this study appears to be T2. With this strategy there was a significant increase in ADG and LMA compared to T1 and C, yet there was not the deleterious effect on tenderness seen in T3. Certainly the argument can be made that T2 is not different from T3 in traits that determine carcass price (HCW, yield grade and USDA quality grade). However, from a consumer aspect, the increased chance of purchasing a less tender steak may ultimately reduce repeat buyers. Implant strategy is a fine balance between optimizing growth and efficiency without compromising quality grade and tenderness.

Chapter Three

EFFECT OF COMBINATION TRENBOLONE ACETATE AND ESTRADIOL IMPLANTS ON BOVINE SATELLITE CELL PROLIFERATION AND DIFFERENTIATION

Abstract

The objectives of this study were to quantify the proportions of proliferating and differentiating muscle satellite cells from implanted and control cattle at different body weights. Holstein steers (n = 24) weighing an average of 199 kg were randomly assigned to an implant treatment or control group and a harvest day of either d 14, d 126 or d 238. Each group consisted of 4 steers. Fourteen days prior to their assigned harvest date, each steer in the implant group received one Component™ TE-S implant (120 mg trenbolone acetate and 24 mg estradiol per implant). Steers were harvested at the Michigan State University Meat Laboratory. Left *semitendinosus* (ST) muscles were removed and used for protein and DNA quantification. Right ST muscles were used for satellite cell isolation. Satellite cells were identified by immunostain for neural cell adhesion molecule (NCAM). Satellite cell proliferation and differentiation were assessed by immunostaining for proliferating cell nuclear antigen (PCNA) and myogenin, respectively. Live weight, ST weight, protein and DNA concentrations increased with age ($P<0.05$) but were not affected by implant treatment. A lower percentage of NCAM+ and PCNA+/NCAM+ cells were observed at d 14 in implanted and control groups, respectively. The percentage of myogenin+ cells was lower in implanted cattle than controls on d 126. No differences were observed in the percentage of PCNA+/NCAM- cells over time or in response to implant treatment. A high proportion of satellite cells (>73%) were PCNA+ and less than 7.7% of satellite cells were myogenin+ at any time

point. These results indicate that the proportions of proliferating and differentiating satellite cells remain relatively constant in growing cattle from 200 to 500 kg body weight, and TBA/E_2 implants have little effect on satellite cell activity 14 d after implantation.

Introduction

Postnatal skeletal muscle growth is associated with an increase in both protein and DNA content (Powell and Aberle, 1975). Because myonuclei cannot synthesize DNA (Stockdale and Holtzer, 1961), satellite cells are required to provide myonuclei to growing skeletal muscle (Moss and Leblond, 1971). Satellite cells proliferate, differentiate and fuse to adjacent muscle fibers when induced by the appropriate stimulation. Subsequently, the nuclei can then become involved in directing muscle protein synthesis (Allen et al., 1983). The ability of satellite cells to proliferate is inversely related with age in rats (Schultz and Lipton, 1982). However, treatment with IGF-I can restore the proliferative capacity of satellite cells in older skeletal muscle *in vitro* (Chakravarthy et al., 2000). Additionally, administration of combination trenbolone acetate and estradiol (TBA/E_2) implants to cattle resulted in increased serum IGF-I levels (Hunt et al., 1991a; Johnson et al., 1998a). These data indicate that the decrease in satellite cell proliferation may be attenuated by treatment with anabolic steroids.

The Food and Drug Administration approved TBA in 1987 for use in implants to increase rate of gain and feed efficiency of cattle. The use of implants composed of TBA and E_2 is currently widespread, due to the additive effects of these compounds on growth rate as TBA/E_2 implants improve average daily gain by 6 to 15%, compared to non-

implanted cattle (Johnson et al., 1996; Foutz et al., 1997; Hermesmeyer et al., 2000). However, the mode of implant action is poorly understood. Thompson et al (1989) demonstrated that serum from Sprague-Dawley rats treated with trenbolone (TBOH) increases rat satellite cell proliferation *in vitro* compared to serum from non-treated rats. Furthermore, satellite cells from TBOH treated rats had increased proliferation compared to satellite cells from controls. Similar results were observed in satellite cells isolated from steers treated with TBA/E_2 implants (Johnson et al., 1998a). Collectively, these data indicate that anabolic implants elicit a response from satellite cells that may contribute to the increased muscle mass observed in treated animals. Therefore, the objectives of this study were to quantify the populations of proliferating and differentiating satellite cells in cattle over time and in response to treatment with a TBA/E_2 implant.

Materials and Methods

Animals. Holstein steers (n = 24; 180kg) were purchased and transported to the Michigan State University (MSU) Beef Cattle Teaching and Research Center. After a 56-d adjustment period, eight steers were randomly assigned a harvest date of d 14, d 126 or d 238 after the initiation of the trial. Within each harvest group, 4 steers were implanted with one Component™ TE-S implant (120 mg trenbolone acetate and 24 mg estradiol per implant; Vet-Life, West Des Moines, IA) 14 d prior to the assigned harvest date and the remaining 4 steers served as non-implanted controls. On the assigned day, steers were harvested at the MSU Meat Laboratory. The right and left *semitendinosus* (ST) muscles were excised quantitatively from the hind limbs, denuded and weighed.

The right ST muscle was placed in cold phosphate buffered saline (PBS) and used immediately for satellite cell isolation. The left ST muscle was cut into 1 to 2 cm^3 cubes, frozen in liquid nitrogen, and stored at -80°C until DNA and protein analysis. All procedures were approved by the MSU Committee on Animal Use and Care (Approval #: 04/99-051-00).

Satellite cell isolation. Satellite cells were isolated using procedures described by Doumit and Merkel (1992). Briefly, ST muscles were excised, trimmed of visible connective tissue and ground in an aseptically prepared grinder. A protease solution containing 0.8 mg/mL Pronase (Sigma Chemical Co., St Louis MO) in PBS was added to ground tissue at a ratio of 40% tissue, 60% protease solution (v/v). Digestion was carried out for 50 min at 37°C in a non-shaking water bath and samples were vortexed at 10 min intervals. Samples were removed from the protease solution by centrifugation at 1000 x *g* for 15 min followed by a wash with PBS. Satellite cells were separated from debris by repeated centrifugation at 300 x *g* for 5 min and subsequently filtered through 500 μm and double-layered 53-μm-mesh nylon cloth. Aliquots of primary muscle isolates were frozen in Dulbecco's Modified Eagle's Medium (DMEM; Gibco BRL, Grand Island NY) containing 20% FBS (Sigma Chemical) and 9% dimethyl sulfoxide (J.T. Baker, Phillipsburg NJ) and stored in liquid nitrogen until use.

DNA and Protein Assays. One gram of ST muscle was homogenized in 25 volumes of extraction buffer (10 mM Tris and 5 mM EDTA, pH 8.0). DNA content was determined using the method of Labarca and Paigen (1980). Briefly, triplicate 100 μL aliquots of muscle homogenate were mixed with 2.9 mL of DNA assay buffer (0.05 M $NaH_2PO{\bullet}H_2O$, 2 M NaCl, 2 mM EDTA and 1 μg/mL Hoechst 33258 reagent (Sigma

Chemical)) and allowed to incubate in the dark for 15 min. Fluorescence was quantified with a Dynaquant fluorometer (Hoefer Pharmacia Biotechnology Inc, San Francisco, CA). Calf thymus DNA (Sigma Chemical) served as a standard. Protein content of the ST muscle was determined using a biuret procedure outlined by Gornall et al. (1948) and modified by Robson et al. (1968). Briefly, triplicate 250 μL aliquots of the muscle homogenate were mixed with 750 μl 1 N NaOH and allowed to incubate at 37°C for 3 to 4 hrs. Four mL of biuret reagent (6 mM $CuSO_4 \bullet 5\ H_2O$, 20 mM $KNaC_4H_4O_6 \bullet 4H_2O$ and 0.75 M NaOH) were added to the samples, which were subsequently incubated in the dark for 30 min. Absorbance of a 250 mL sample aliquot was read at 540 nm in a microplate reader (Versamax, Sunnyvale, CA). Bovine serum albumin (Sigma Chemical) served as the protein standard.

Hybridoma culture. The 5.1H11 cell line produces a monoclonal antibody against neural cell adhesion molecule (NCAM) and was developed by Dr. Helen Blau at Stanford University School of Medicine, Stanford CA and Dr. Frank Walsh at the United Medical and Dental Schools of Guy's and St. Thomas' Hospitals, London, United Kingdom. The F5D cell line produces a monoclonal antibody against myogenin and was developed by Dr. Woodring Wright at the University of Texas Southwestern Medical Center, Dallas TX. Both cell lines were obtained from the Developmental Studies Hybridoma Bank maintained by the University of Iowa, Department of Biological Sciences (Iowa City, IA) under the auspices of the National Institute of Child Health and Human Development (NICHD).

The 5.1H11 hybridoma was cultured as described by Mesires (2000). Briefly, 5.1H11 hybridoma cells were thawed and seeded in 75 cm^2 flasks in DMEM with 25 mM

Hepes (Gibco BRL), 2 mM L-glutamine, 1 mM sodium pyruvate, 1 mM nonessential amino acids (Gibco BRL), 1% antibiotic/antimycotic (AB/AM; Sigma Chemical) and 10% FBS for 3 d. Cultures were expanded by a 4:1 dilution (fresh media to cell suspension) and subsequent transfer to new flasks as necessary. Expansion was repeated every 48 h until a desired volume of supernatant was achieved, at which time the cultures were allowed to remain undisturbed for 12 to 14 d. Supernatant was collected after centrifugation at 2000 x *g* for 15 min and stored at -20°C.

The F5D hybridoma was cultured as described in Mesires (2000). Briefly, F5D hybridoma cells were thawed and plated in 75 cm^2 flasks in RPMI (Gibco BRL), 10% FBS and 0.025% gentamycin (Sigma Chemical) for 3 d. Expansion and supernatant collection were performed as described for the 5.1H11 hybridoma.

Neural Cell Adhesion Molecule Validation. Bovine satellite cells were plated at 2.5, 5.1 and 10.2 cells per cm^2 on gelatin coated 100 mm culture plates in growth media consisting of DMEM, 10% FBS, 0.5% AB/AM and 0.1% gentamycin. Cells were allowed to grow undisturbed for 1 wk. Medium was then replaced with differentiation medium consisting of DMEM with 2% horse serum. Cells were incubated for an additional 2 d, with fresh differentiation medium supplied every 24 h. Cells were washed once with PBS containing 1% goat serum (blocking solution), incubated in 5.1H11 hybridoma supernatant for 30 min then washed three times with blocking solution. Cells were then incubated with 0.5 μg/mL biotinylated goat-anti-mouse IgG_1 (Caltag Laboratories, Burlingame, Ca) for 30 min and washed as before. Finally, cells were incubated with an extravidin alkaline phosphatase (Sigma Chemical) and visualized with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium as a substrate to produce a

dark blue reaction product. Colonies positive for NCAM staining were identified without magnification on a gel illuminator. Subsequently, cells were fixed in ethanol and counterstained with Giemsa to identify all colonies in the same manner. Colonies were scored based on the presence or absence of myotube development on a Zeiss inverted microscope (Oberkochen, West Germany) at 400x magnification.

Satellite Cell Preparation and Immunostaining. Bovine satellite cells were separated from debris utilizing a Percoll gradient as described by Yablonka-Reuveni (1989). Briefly, 2 mL of 60% Percoll (Sigma Chemical) in DMEM was added to a 30 mL corex tube. Eight mL of 20% Percoll in DMEM was layered upon the 60% solution. Primary cell isolates derived from 3.5 g of muscle tissue were loaded onto the Percoll gradient. Gradients were centrifuged at 15,000 x *g* for 5 min. The interface between the 20% and 60% Percoll solutions was harvested, diluted and centrifuged at 300 x *g* for 15 min to pellet cells. The pellet was resuspended in 200 µL DMEM and cells were aliquoted onto two separate 12 mm diameter coverslips (Fisher Scientific, Itasca, IL) placed in a 24-well plate. Cells were allowed to absorb to the coverslips for 45 min.

Cells were immunostained for NCAM, proliferating cell nuclear antigen (PCNA) and myogenin using the methods of Mesires and Doumit (2002) with the following revisions: Blocking solution consisted of 1% goat serum in PBS and the concentration of biotinylated goat-anti-mouse IgG_1 (Caltag Laboratories, Burlingame, Ca) was 0.5 µg/mL. Briefly, cells absorbed to coverslips were incubated in blocking solution to minimize nonspecific antibody binding. All incubations and washes were performed in volumes of 0.5 mL. Cells were incubated in 5.1H11 hybridoma supernatant for 30 min and washed three times in blocking solution. Cells were then incubated with biotinylated goat-anti-

mouse IgG_1 and the washing series was repeated. Finally, cells were incubated with a 1:200 dilution of ExtrAvidin-tetramethylrhodamine isothiocyanate (TRITC; Sigma Chemical Co., St Louis, MO) in blocking solution for 30 min followed by a wash. Cells were fixed in 1% formalin for 5 min followed by exposure to –20°C methanol for 5 min. After a wash series, one coverslip from each sample was immunostained for PCNA, while the other was stained for myogenin.

Immunostaining for PCNA. Bovine satellite cells were incubated with 1 μg/mL anti-PCNA monoclonal antibody (PC10; BioDesign, Caco, ME) in blocking solution overnight in a humidified chamber at 4°C. Cells were washed and subsequently incubated in 0.8 μg/mL goat anti-mouse IgG_{2a} conjugated to fluorescein isothiocyanate (FITC; Caltag laboratories). Coverslips were washed and mounted onto microscope slides using Vectashield mounting media (Vector, Burlingame, CA) containing 4',6-diamidino-2-phenylindole (DAPI) to counterstain DNA. Coverslips were sealed with nail polish and viewed. The number of total cells (DAPI), total satellite cells (NCAM+), total proliferating cells (PCNA+) and total proliferating satellite cells (NCAM+ and PCNA+) were counted by two independent evaluators blinded to the identity of the sample. A minimum of 88 cells per animal were analyzed for each animal.

Immunostaining for myogenin. Bovine satellite cells were incubated in F5D hybridoma supernatant overnight in a humidified chamber at 4°C. Cells were washed, then incubated for 30 min with a 1:100 dilution of goat anti-mouse IgG_1 conjugated to FITC. After a wash series, coverslips were mounted onto coverslides using mounting media containing DAPI and sealed with nail polish prior to being viewed. Two

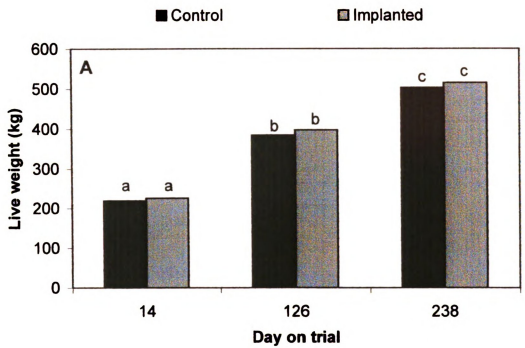
independent evaluators enumerated total, NCAM+ and myogenin-positive (myogenin+) cells. A minimum of 70 cells per animal were analyzed for each animal.

Statistics. Data were analyzed using the general linear model procedures of SAS (SAS Inst. Inc., Cary, NC). Percentage data were converted to a decimal value and transformed as the inverse sine of the square root. For the analysis of all data, the model included harvest group, treatment, and the harvest group by treatment interaction. Pair-wise comparisons within treatment or within harvest group were made. Data are reported as least significant means (LSmeans) with a pooled standard error of the mean (SEM).

Results and Discussion

Live weight and muscle characteristics. Live weight increased with age. *Semitendinosus* weight increased in proportion to live weight. Implant treatment had no effect on live weight or ST weight (Figure 1A). An effect of implant on live weight or ST weight was not expected 14 d after implant. Johnson et al. (1996) demonstrated that 40 d after treatment with a TBA/E_2 implant, live weight and *longissimus* muscle area were not different, although differences were observed at d 115. *Semitendinosus* protein content increased in proportion to DNA content although DNA accretion appeared to plateau at d 126 and was not different from the DNA content on d 238 (Figure 1B). This may be attributed to the steers approaching the end of the rapid growing phase (Di Marco et al., 1987). Implant treatment had no effect on protein accretion although implanted cattle tended to have higher DNA content ($P = 0.06$) than control cattle at d 14. In the *longissimus* muscles of growing hogs, total muscle protein parallels an increase in muscle mass and age has little effect on the percentage of muscle protein (Harbison et al., 1976).

Figure 1. Live weight and *semitendinosus* weight of control and implanted steers. Panel A: Live weights on d 14, 126 and 238 of the trial. Pooled standard error of the mean (SEM) for live weight is 11.19 kg. Panel B: *Semitendinosus* (ST) weights on d 14, 126 and 238 of the trial. Pooled SEM for ST weight is 85.2 g. Different labels inidicate that means within treatment are different.


Control
Implanted
A
Live weight (kg)
600
500
400
300
200
100
0
a
a
b
b
c
c
14
126
238
Day on trial

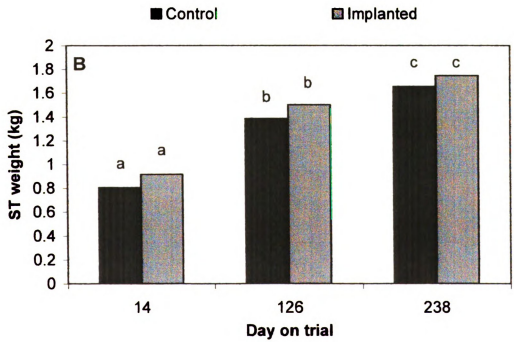
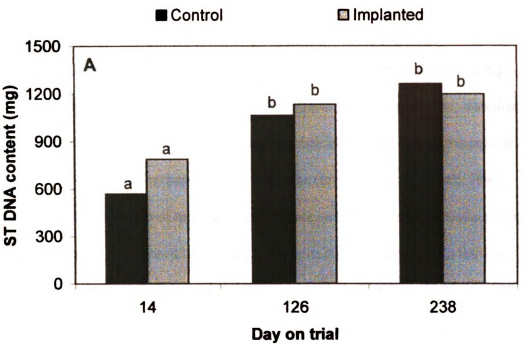
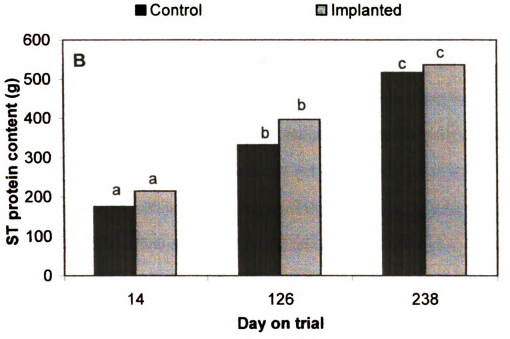

Control
Implanted
B
ST weight (kg)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
a
b
b
c
c
14
126
238
Day on trial

Figure 2. DNA and protein content of *Semitendinosus* muscle from control and implanted steers. Panel A: *Semitendinosus* DNA content on d 14, 126 and 238 of the trial. Pooled SEM for ST DNA content is 78.0 mg. Panel B: *Semitendinosus* protein content on d 14, 126 and 238 of the trial. Pooled SEM for protein content is 16.9 g. Different labels indicate that means within treatment are different.


Control
Implanted
A
ST DNA content (mg)
1500
1200
900
600
300
0
a
a
b
b
b
b
14
126
238
Day on trial


Control
Implanted
B
ST protein content (g)
600
500
400
300
200
100
0
a
a
b
b
c
c
14
126
238
Day on trial

Furthermore, an increase in muscle mass is also highly correlated to an increase in myonuclei as demonstrated by Powell and Aberle (1975). The numerical increase in DNA content in young cattle may be the result of activation of satellite cells by the implants. This increase is only observed in the young cattle and may be due to a higher percentage of muscle nuclei that are satellite cells capable of DNA synthesis. Satellite cells consist of about 33% of total muscle nuclei at birth in rats and that percentage drops to a maintenance level of a few percent after 2 months (reviewed by Allbrook, 1981). This decline in the proportion of muscle nuclei that are satellite cells likely occurs over a longer period in cattle as opposed to rats and is supported by the observation that the apparent yield of satellite cells per gram was much greater at d 14 than d 126 or d 238 (unpublished observations).

Neural cell adhesion molecule validation. Neural cell adhesion molecule has been validated as a marker for human (Walsh and Ritter, 1981) and pig (Blanton et al., 1999) satellite cells. Clonal analysis of primary bovine satellite cells revealed that 61% of the NCAM positive colonies showed the development of myotubes, while the remaining 39% did not have myotube development. No myotube development was observed in the 10 NCAM negative colonies. These data indicate that a majority of cells expressing NCAM are myogenic, whereas cells that do not express NCAM are not myogenic. Thus, the NCAM+ cell population was considered to be satellite cells. This study differs from that of Baroffino et al. (1993) and Blanton et al. (1999) in that a clonal analysis was used rather than flow cytometry.

Immunostaining for NCAM, PCNA and myogenin. Serum from rats treated with TBOH increased proliferation of rat satellite cells compared with control rat serum.

Serum concentration of estradiol peaks around d 12 after implantation of cattle with a TBA/E_2 implant (Lee et al., 1990), while IGF-I peaks around d 6 after implantation (Johnson et al., 1998a). Furthermore, satellite cells isolated from steers about 35 d after implanting with TBA/E_2 had a shorter lag phase prior to the initiation of proliferation *in vitro*. These data indicate that satellite cell activity is changed in response to an implant in a relatively short period of time. Therefore in this study satellite cells were isolated from Holstein steers 14 d after implanting with a TBA/E_2 implant.

Proliferating cell nuclear antigen was used to detect proliferating cells in the current study. The expression of PCNA is limited to the S phase of the cell cycle (reviewed by Baserga, 1991) and is a useful marker to detect satellite cell entry into the cell cycle prior to an increase in cell number (Johnson and Allen, 1993). The use of PCNA as a cell proliferation marker offers several advantages over other methods to assess cell proliferation. PCNA visualization minimizes the use of toxic or radioactive reagents such as BrdU and H^3-thymidine that require invasive administration techniques and expensive disposal. However, PCNA does not allow for the quantification of low level cell replication (Jones et al., 1993).

Myogenin was used as a marker for cell differentiation. Myogenin expression is up regulated early in the differentiation process and precedes the expression of the cell cycle inhibitor, p21 (Andres and Walsh, 1996). Myogenin positive cells are capable of synthesizing DNA until up regulation of p21 expression (Andres and Walsh, 1996). Thus, myogenin and PCNA can be co-expressed, explaining why in some cases the sum of the percentages of PCNA+ and myogenin+ cells within the population of NCAM+ cells is greater than 100%. Therefore, the data do not reflect absolute numbers of cells,

but are an index of the relative populations of proliferating and differentiating satellite cells.

The percentage of NCAM+ cells was not different between implanted and control muscle isolates (Figure 3). A higher percentage ($P<0.05$) of NCAM+ cells was present in d 126 than d 14 in muscle isolates from implanted cattle, however this difference was not seen in nonimplanted cattle. Similar percentages of NCAM+ cells were observed on coverslips stained for the quantification of PCNA or myogenin (data not shown).

In satellite cell isolates from the ST of growing pigs, Mesires and Doumit (2002) demonstrated that the percentage of NCAM+ cells decreases from 1 wk to 7 wk and then remains unchanged through 21 wk. Age associate changes in the percentage of NCAM+ cells were not observed for control steers. Within the implanted groups, the percentage of NCAM+ cells was lower at d 14 when compared to d 126. These data are associated with a numerical increase in proliferating non-myogenic cells (PCNA+/NCAM-) from implanted cattle on d 14 relative to controls, which may result in a dilution of NCAM+ cells. Thompson et al. (1989) demonstrated that TBOH, alone or in combination with IGF-I, does not affect satellite cell proliferation or differentiation directly *in vitro*. However, satellite cells isolated from TBOH treated Sprague-Dawley rats were significantly more responsive to IGF-I resulting in increased total nuclei with an increased fusion percentage (Thompson et al., 1989). Therefore, the reduced percentage of NCAM+ cells may be the result of increased proliferation of both myogenic and nonmyogenic cells with increased differentiation and fusion of satellite cells, and these effects are age dependent.

The percentage of proliferating satellite cells (PCNA+/NCAM+) was not affected

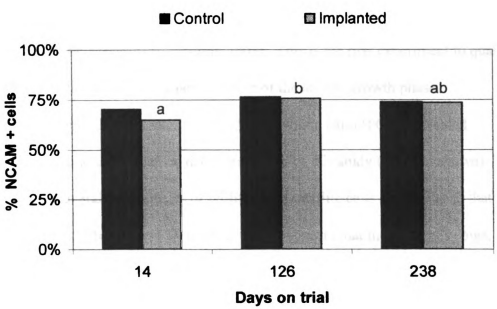


Figure 3: Changes in the percentage of NCAM positive cells (satellite) isolated from the right *semitendinosus* of implanted and nonimplanted steers. The pooled SEM is 3.37%. Different superscripts indicate that means are different within treatment ($P > 0.05$). No differences within day were observed ($P < 0.05$)

by treatment, although the percentage of PCNA+/NCAM+ cells tended to be higher in isolates from implanted steers relative to controls at d 14 ($P = 0.07$; Figure 4). Within the control groups, the percentage of PCNA+/NCAM+ cells was lower in d 14 muscle isolates than that found at d 126 or d 238 ($P < 0.05$). A lower percentage of proliferating satellite cells at d 14 relative to d 126 and d 238 is contrary to several reports that show that satellite cell proliferation decreases with age (Schultz and Lipton, 1982; Yablonka-Reuveni et al., 1999; Mesires and Doumit, 2002). This is the first experiment to quantify bovine satellite cell activity over a large portion of the animal growth phase. Quantification of proliferating satellite cells from a young calf (100 kg) revealed percentages similar to those seen on d 126 and d 238 of this study (data not shown). Furthermore, the overall percentages of proliferating satellite cells are similar to that reported for the pig (Mesires and Doumit, 2002). It appears that in cattle, as in pigs, a high proportion of satellite cells are proliferating throughout the growing phase.

In the analysis of myogenin+/NCAM+ cells, a significant interaction ($P < 0.05$) existed between treatment and harvest group (Figure 5). A decrease in the percentage of myogenin+/NCAM+ was observed in isolates from implanted cattle at d 126 when compared to controls ($P < 0.05$). Within the implanted groups, a lower percentage of myogenin+/NCAM+ cells were observed in d 126 isolates than d 14 ($P < 0.05$). A general decrease in myogenin+ satellite cells agrees with data shown by Mesires and Doumit (2002). However, the differences observed at d 126 are difficult to explain. The harvest group x treatment interaction may be a random effect associated with only looking at two treatments with three harvest groups. In addition, the percentages of differentiating satellite cells (3.8% to 7.7%) were much lower than observed in the pig

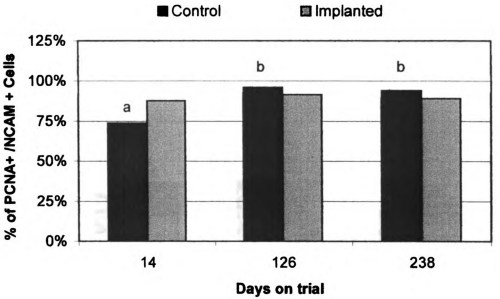


Figure 4: Changes in the percentage proliferating satellite cells (PCNA+/NCAM+) isolated from the right *semitendinosus* muscle of implanted and nonimplanted steers. The harvest group x treatment interaction was $P = 0.08$. The pooled SEM is 6.56%. Different superscripts indicate that means are different within treatment ($P > 0.05$). No differences within day were observed ($P < 0.05$)

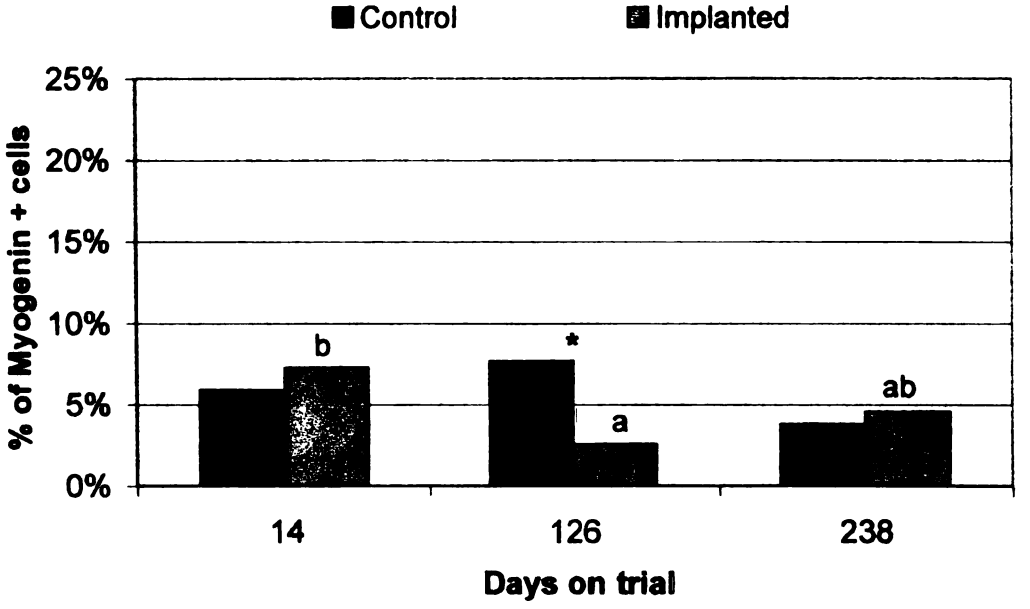


Figure 5: Changes in the percentage of differentiating satellite cells (cells expressing myogenin) isolated from the right *semitendinosus* muscle of implanted and nonimplanted steers. The harvest group x treatment interaction was P = 0.05. The pooled SEM is 3.10%. Different superscripts indicate means are different within treatment (P > 0.05). The asterisks indicates that means are different between control and implanted treatments within harvest group (P>0.05).

(Mesires and Doumit, 2002)which ranged from 9 to 31%. These differences may be attributed to the relative physiological maturity of the animals used in each experiment.

Finally, no differences were observed in the percentages of PCNA+/NCAM- cells within harvest group or treatment although there appears to be a general trend for increased proliferation over time (Figure 6). These data corroborate the findings of Mesires and Doumit (2002) who showed an increase in the percentage of proliferating cells that are nonmyogenic. Furthermore, Vandenburgh et al. (1984) showed serum from older mice increased the percentage of fibroblast-like cells in cultures of chicken muscle cells compared to extracts form younger mice.

Conclusions

The weight of the ST muscle and the amount of protein and DNA parallel increases in live weight. Satellite cells maintain a constant level of proliferation in cattle weighing 200 to 500 kg. The percentage of myogenin+ satellite cells tends to decrease with age while the percentage of non-myogenic cells tends to increase. Implant treatment has little effect on satellite cell activity 14 d after treatment with a combination TBA/E_2 implant. Changes in satellite cell activity during growth in cattle may be more subtle than what has been previously measured for other species. Detection of developmental changes in satellite cell activity may require the analysis of both younger and older cattle.

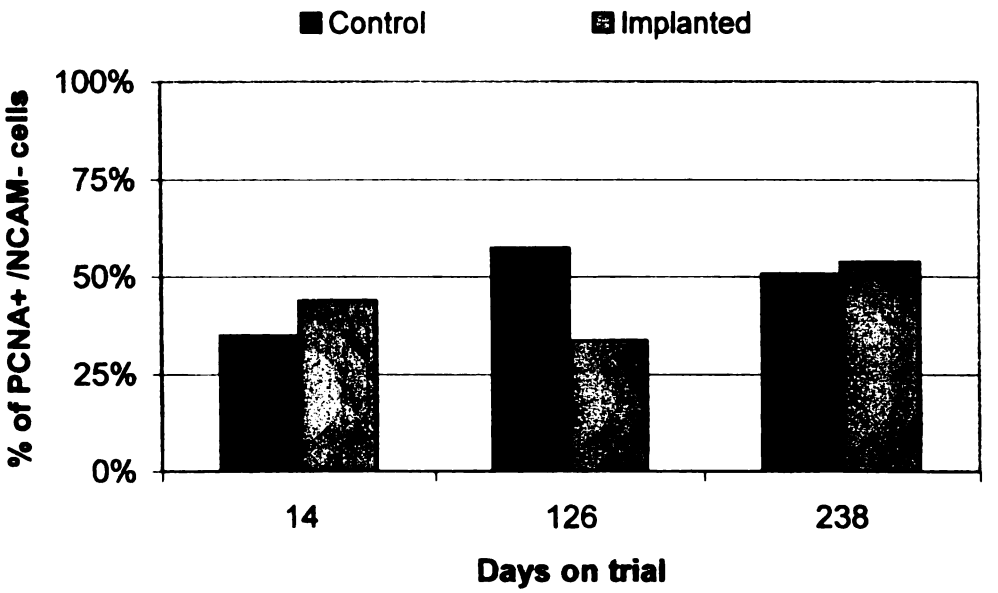


Figure 6: Changes in the percentage of proliferating nonmyogenic cells (both PCNA positive and NCAM negative) isolated from the right *semitendinosus* muscle of implanted and nonimplanted steers. The pooled SEM is 9.06%. No differences within day or within treatment were observed ($P < 0.05$)

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Literature Cited

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