THE EFFECTS OF INTAKE ON STEERS ADMINISTERED ANABOLIC IMPLANTS

by

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Abstract

The objectives of this study were to examine the effect of anabolic implants on nutrient balance, metabolic status, and growth factors in animals consuming nutrients either adequate or inadequate to support growth. Sixteen crossbred steers (BW 293 ± 19.3 kg) were trained to individual Calan gates, and randomly assigned to one of four treatments in a 2x2 factorial arrangement: (1) administration of an anabolic growth implant, and fed a moderate energy starting cattle diet at 2.0 × maintenance; (2) implant administration, and fed the same starting diet at 1.0 × maintenance; (3) no implant, and 2.0 × maintenance; (4) no implant and 1.0 × maintenance diet. Cattle were implanted with RevalorXS, containing 200 mg TBA and 40 mg estradiol. Animals were weighed on d 0, 14, and 28, with total gain, ADG, and feed efficiency determined at each time point. Blood samples were taken from each animal at d 0, 14, and 28 and used in determining serum concentrations of IGF-1 and plasma urea nitrogen (PUN). Serum collected on d 14, and 28 was applied to satellite cells (previously isolated from non-study steers and frozen). Protein abundance of myosin heavy chain (MYH; d 0, 14, and 28), phosphorylated extracellular signal related kinase (pERK; d 0 and 28), and phosphorylated mammalian target of rapamycin (pmTOR; d 0 and 28) was analyzed in differentiated satellite cells to determine effects of implant, intake, and their interaction (applied via the serum). There was a significant effect of diet on weight ($P < 0.0001$). There was a tendancy for an interaction between diet and implant on PUN ($P = 0.09$). Only diet had an effect on IGF-1 levels ($P < 0.001$). Implant increased MYH abundance ($P < 0.01$), and the abundance of pERK ($P < 0.01$). At high intake, implant increased abundance of pmTOR ($P = 0.02$) but had no effect on pmTOR at restricted intake ($P = 0.21$; interaction $P < 0.01$). These preliminary results show that implantation, which

has previously been shown to improve gain, ADG, and feed efficiency, may not be as beneficial in cattle fed a restricted diet.

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Dedication

I would like to dedicate this thesis to my parents, Larry and Teresa Lee, and to my brother, Brian Lee. There are many people in my life who have been there for me through difficult times, but none moreso than these. I could not and would not ask for a better support group. I hope that the accomplishments that they have helped me achieve will make them proud.

Chapter 1 - The Effects of Nutrient Intake on Steers Administered Anabolic Implants: A Review of the Literature

Introduction

With the expansion of the human population from approximately 2.5 billion people in 1950 to almost 7 billion people today (U.S. Census Bureau, 2011), there is an obvious need to find new and different ways to increase the amount of safe and nutritious food to support this ever-growing population. Numerous research trials have been conducted to advance our understanding of the growth and development of plants and animals grown for food purposes, and especially that of animals bound for red meat consumption. In turn, our understanding of the physiological processes of growth has expanded. Knowledge of these processes has led to great advances in the production of meat for the world's population. These advances in production will need to continue if we will feed the 9 billion people who are expected to populate our planet by the year 2050 (U.S. Census Bureau, 2011).

One of these advances in production could include increasing the efficiency with which animals produce red meat, or, in essence, grow. Animals must grow larger in a shorter amount of time to increase this efficiency. Efficiency of growth in animals can also be increased if the growth can be obtained with less feedstuffs consumed (Hutcheson *et al*, 1997). Not only do these increases in efficiency help us to produce more red meat in a shorter amount of time, they also help to decrease the amount of inputs that must be utilized to produce that amount of meat. It has been determined that the use of anabolic steroid implants offers the biggest return on investment related to increasing efficiency, outside of providing the most adequate nutrition to beef cattle (Montgomery *et al*, 2001). Anabolic steroid implants have been shown to increase

average daily gains 10 to 21%, and improve feed:gain ratios by 6 to 14% in feedlot cattle (Johnson *et al*, 1996, Duckett & Owens, 1997, Duckett & Andrae, 2001, Bruns *et al*, 2005). Such responses leave little room to argue the use of implants in the beef cattle industry for improved efficiency and growth.

However, this drive for improved efficiency and growth can come with some negative consequences. Many cattle arrive at feedlots in a state of stress because they are not yet accustomed to the environment, feeding routine, a new social order, and many other changes encountered at this time. Stress levels can be increased if cattle have been hauled long distances, or have recently been weaned from the cow (Loerch & Fluharty, 1999). These cattle are at higher risk for increased morbidity and mortality rates, and for decreased performance in the feedlot (Loerch & Fluharty, 1999). Many of these high-risk cattle have reduced intakes for up to three weeks after arrival at the feedlot (Hutcheson & Cole, 1986). The stress of these events can lead to immunocompromised cattle, which lead to an increased risk of bovine respiratory disease (BRD) (Blecha *et al*, 1984), the number one cause of death in cattle and calves in the United States (NAHMS, 1994).

In humans and other non-ruminants, stress and/or infection (such as BRD in cattle) can lead to a hypermetabolic state (Cole *et al*, 1986). This condition is characterized by weight loss, increased resting metabolic rate, and increased muscle protein catabolism (Cole *et al*, 1986). Cattle suffering from bovine respiratory disease often have decreased feed intake, weight loss, and decreased body condition (Cole *et al*, 1986). Ruminants have a greater capacity to store and utilize nutrients because of their larger and more diverse digestive tracts; however, this weight loss and decrease in body condition can be comparable to the hypermetabolic state that develops in non-ruminants (Cole *et al*, 1986). In such high-risk cases, implanting cattle upon arrival at the

feedlot has been suggested to be detrimental to their performance, possibly turning that anabolic effect into a catabolic state in the muscle tissue.

Anabolic Steroid Implants

The utilization of steroid hormones in anabolic implants dates back to 1947, when Hereford heifers were experimentally implanted with diethylstilbestrol (DES), a form of synthetic estrogen (Raun & Preston, 2002). This initial study revealed that steroid implants increased rate of gain (approximately 15%), and improved feed efficiency (approximately 10%) in cattle, and steroid implants have been studied and documented ever since. The USDA approved the use of DES in feedstuffs in 1954, and as an injectable implant in 1957 (Raun & Preston, 2002). The use of DES was prohibited in cattle, in feedstuff and injectable form, by the FDA in 1973 (Raun & Preston, 2002). However, other growth promotants containing estrogen analogs, progesterone, and trenbolone acetate were developed, and are approved by the FDA. Today, over 90% of all feedlot cattle are implanted with some form of anabolic implant at least once, and usually more than once, before slaughter (Dikeman, 2007).

There are a number of estrogenic, androgenic, and combination implants approved for use in beef cattle today (Table 1). According to Dikeman (2007), estrogenic compounds are generally more effective in steers, while androgenic compounds are usually more effective in heifers. It has been recognized that combination implants containing both trenbolone acetate (TBA—an androgen analog) and estradiol are the most effective growth promoters in cattle (Duckett & Andrae, 1997, Duckett & Andrae, 2001, Foutz *et al*, 1990, Johnson *et al*, 1996, Thomson *et al*, 1996).

Estrogenic and androgenic implants increase growth by increasing protein accretion in muscle tissue (Foutz et al, 1997). The increased muscle tissue protein accretion results in

increased average daily gain (ADG) and increased hot carcass weight in cattle (Duckett & Andrae, 2001). Cattle implanted with steroid hormones also have improved feed efficiency (Guiroy et al, 2002). As previously stated in this review, anabolic implants have been shown to increase average daily gains 10 to 21%, and improve feed:gain ratios by 6 to 14% in feedlot cattle (Johnson *et al*, 1996, Duckett & Owens, 1997, Duckett & Andrae, 2001, Bruns *et al*, 2005) compared to cattle not treated with steroid implants.

Steroid implants have been determined to aid the industry in producing an increased amount of lean beef with a reduced amount of feed utilized. However, there have been some industry concerns about the impact of implants on carcass traits (Foutz *et al*, 1997, Morgan *et al*, 1997, Dikeman, 2007). Decreased marbling, decreased palability of beef and decreased tenderness of meat, are producer concerns when using steroid implants in cattle (Foutz, *et al*, 1997, Morgan *et al*, 1991). Nichols *et al.* (2002) reviewed the effects of steroids implants on beef tenderness. In their review, they found that only three out of 19 studies showed negative effects of steroid implants on beef tenderness as determined by Warner-Bratzler shear force scores compared to beef from non-implanted cattle. It should be noted that two studies actually reported an increase in tenderness of beef from implanted cattle compared to that from non-implanted cattle. Because of the differences in the results of these studies, it must be considered that there may be no difference in tenderness, and these results showing increased or decreased tenderness could be due to chance. Such pros and cons of the use of anabolic implants in beef production have led to the use of multiple implant strategies throughout the beef production circuit in an effort to maximize the performance of different cattle types in different environments, with different resources, throughout the country.

There are many different steroid implant strategies used by beef producers today. Cattle can be implanted as suckling calves, stocker cattle, yearlings, and at many other points in the marketing process. Different steroid implant strategies can be used for increased production in heifers versus steers (Montgomery *et al.* 2001). It has been shown that implanting steers with an androgenic, estrogenic or acombination (androgen + estrogen) implant has a positive effecs on body weight, ADG, and F:G mentioned previously in this paper (Foutz *et al*, 1997, Duckett & Owens, 1997). However, when implanting heifers, a meta-analysis done by Wileman *et al.* in 2009 showed that implanting improved only average daily gain, and not feed:gain or dry matter intake. However, this could possibly be attributed to different diets being fed among the studies included in this meta-analysis. According to Duckett and Andrae (2001), a strategy that utilizes a combination implant at two different times while the cattle are in the feedlot has been reported as the most effective strategy when increased average daily gain and feed efficiency are desired.

It is common strategy to implant cattle upon arrival at the feedlot, and then at approximately 100 days prior to slaughter. In 2007, a new long-lasting steroid implant was approved by the FDA. This implant eliminates the need to re-implant cattle at 100 days prior to slaughter, and therefore eliminates a potential source of stress for the cattle, and expense for the feedlot. Revalor XS (Merck, Summit, NJ) consists of 200 mg of TBA and 40 mg of estradiol per dose. The implant is a time-release product that dispenses a regular dose of steroid hormones, based on the plastic coating technology that surrounds the implant (Intervet Schering-Plough Animal Health). Such a mechanism of delivery may reduce labor costs and stress due to processing, while still providing the necessary implant for the desired improvement in performance.

Skeletal Muscle Growth and Muscle Satellite Cells

The growth of skeletal muscle tissue involves a unique and complex series of events. The basic cellular unit of muscle tissue is the muscle fiber. Most muscle fibers are formed during embryonic growth, and the formation of fibers does not continue appreciably after birth in most mammals. Postnatal skeletal muscle growth, or hypertrophy, can be attributed to both an increase in diameter and length of the muscle fibers. Skeletal muscle hypertrophy is regulated by at least three major molecular processes: satellite cell activity, gene transcription and protein translation (Machida & Booth, 2004).

Most muscle fibers and the nuclei within them are post-mitotic after birth, resulting in the inability to divide (Allen *et al*, 1979). A correlation between the amount of DNA in the muscle cells and muscle weight during growth was shown by Trenkle *et al* (1978). Continued growth of muscle fibers can be accomplished with hypertrophy of the cells, but this hypertrophy must be sustained by an external source of DNA (Moss & LeBlond, 1970). Multiple studies have shown that, postnatally, there is an increase in the amount of DNA content in the muscle fibers of various mammals (Winick & Noble 1966, Moss 1968, Johns and Bergen 1976,). This external source of DNA is the muscle satellite cell (Moss & LeBlond, 1970).

Satellite cells were discovered by Alexander Mauro in 1961, using electron microscopy of the peripheral region of the skeletal muscle of frogs. At the time of his discovery, Mauro hypothesized on the purpose of these cells, but it wasn't until the 1970's that the true function of muscle satellite cells was discovered. In 1970, Moss and LeBlond concluded that the satellite cells associated with skeletal muscle fibers are capable of undergoing mitosis, and this mitosis is the source of DNA for the true muscle fibers. This conclusion was reached after thymidine-3H was incorporated into satellite cell nuclei and then subsequently found in the nuclei of the

associated true muscle fibers. After the incorporation of the satellite cell into the true muscle fiber, the satellite cell nuclei lose their capacity to proliferate (Moss and LeBlond, 1971).

It is this process of satellite cell proliferation and the subsequent incorporation of external DNA into true muscle fibers that allows postnatal hypertrophy of muscle tissue (Johnson *et al*, 1998). However, satellite cells are not in a continuous state of proliferation. They reside in a state of quiescence, and must be stimulated by hormones to progress through the cell cycle. It has been found that the progression factor insulin-like growth factor 1 (IGF-1) is a stimulant of this proliferation (Johnson *et al*, 1998).

Signalment of Skeletal Muscle Growth and the Effects of Implantation

IGF-1 is a peptide hormone synthesized and secreted mainly from the liver, that mediates the growth-promoting actions of growth hormone (Hong & Forsberg, 1994). The anabolic effects of IGF-1 on skeletal muscle cells and satellite cells include stimulation of amino acid uptake, protein synthesis, glucose uptake, DNA and RNA synthesis, cell proliferation, and cell differentiation (Hong & Forsberg, 1994, Machida & Booth, 2004).

Johnson and others (1996) concluded that circulating levels of IGF-1 were increased in steers implanted with a combined TBA/E2 implant as compared to control steers, given no implant. It has also been shown that a combined TBA and estradiol implant can increase the time in which satellite cells become activated in culture (Johnson *et al*, 1998). An increase in IGF-1 production and satellite cell activation of implanted cattle contributes to the positive effects of implantation on enhancing protein accretion in growing true muscle cells. This has led to the inference that implants directly or indirectly activates quiescent muscle satellite cells in the body, therefore enhancing growth (Johnson *et al*, 1998).

This muscle growth, or more specifically, protein synthesis, can be indirectly measured *in vivo* by the determination of the amount of nitrogen in the plasma of these animals (Sastry & Kravtchenko, 1980). Steroid implants are not involved directly in the deposition of protein in muscle cells, but rather in the signalment of the body to increase protein synthesis. Therefore, the amount of nitrogen in an animal's plasma can provide useful information to help determine whether that synthesis is in fact happening (Thomson *et al*, 1996).

There are three ways in which the amount of nitrogen in the plasma of an animal can be changed—changes in protein synthesis, changes in protein degradation, or both. Anabolic growth implants affect the element of protein synthesis in the case of beef cattle. These implants increase the utilization of nitrogen for the use of the aforementioned synthesis of protein (Thomson *et al*, 1996). Because more of the body's nitrogen store is being used in those implanted animals, it has been shown that urea nitrogen levels in the plasma of implanted steers versus non-implanted steers is lower (Thomson *et al*, 1996).

It has been hypothesized that the synthesis of proteins could be in response to the activation of certain pathways regulated by activation factors, specifically mammalian target of rapamycin (mTOR) and extracellular signal-related kinases (ERK). It has been hypothesized that the administration of anabolic implants increases the activation of these pathways, or the amount of signaling kinases that control these pathways (Figure 1.1). The phosphorylation of these signalment proteins may indicate the "turning on" or "turning off" of certain cellular pathways that increase muscle protein synthesis, and therefore, muscle growth. The ERK protein plays an important role in cell proliferation and differentiation (Lai *et al*, 2001). mTOR has been shown to play an essential part in the regulation of many cellular activities, including transcription, translation, cell size, and protein stability (Corradetti & Guan, 2006). To help

regulate these processes, mTOR integrates information about nutrient status, energy status, and many growth factors (Sorbassov *et al*, 2005). It is because of this integration and its effects on so many different cellular activities that mTOR could be helpful in determining the effects of implantation and nutrient availability in beef cattle.

Stress Experienced by Newly-arrived Cattle in the Feedyard

As cattle move through the normal livestock marketing system, they are subject to a number of different stressors (Hutcheson & Cole, 1986). Such stressors include weaning, feed and water deprivation, crowding, and introduction to infectious agents (Hoerlein & Marsh, 1957). After arrival at the feedlot, cattle can be exposed to other stressors such as processing, including castration, dehorning, vaccination, deworming, and implantation (Hutcheson & Cole, 1986). These stressors can be characterized in the animal by transient endocrine responses, alterations in products of energy and protein metabolism, changes in appetite and growth rate, compromised digestion and rumen function, and challenges to an animal's immune system (Loerch & Fluharty, 1999).

Transportation stress can have a huge impact on newly arrived cattle. Shrink, or loss of body weight, during transportation can be attributed to dehydration, rumen stasis, the depletion of nutrients in the digestive tract due to urine and fecal loss, and tissue loss due to stress (Hutcheson & Cole, 1986). Overcrowding, poor air quality, and poor sanitation during long hauls can exacerbate these problems (Loerch & Fluharty, 1999).

Management is critical to optimize cattle performance when stressed cattle arrive at the feedlot. It can take up to 4 days before healthy cattle start eating, depending on previous environment and length of transport, and even longer for cattle that have been affected by disease (Hutcheson & Cole, 1986). Even after these cattle start to eat, appetite may be decreased for up

to 1 to 3 weeks after arrival (Hutcheson, 1980). This nutritional deficiency can interact with the stress of weaning, comingling, and/or processing, along with exposure to new infectious agents to cause a large compromise in immune function (Galyean, *et al.* 1999).

Numerous studies have shown that the response and performance of feedlot cattle during the overall feeding period is affected by their health and performance response during the receiving/starting period (Roeber *et al*, 2001, Owens & Gardner, 1999, Snowder, 2007, Garcia *et al*, 2010). Thus, the receiving period plays a crucial role in the economic outcome of cattle feeding (Hicks, 2010).

Bovine Respiratory Disease in Newly-arrived Feedlot Cattle

Bovine Respiratory Disease continues to be the most significant health problem in newly weaned and/or received feedlot cattle (Duff & Galyean, 2007). According to Snowder (2007), BRD is the most costly feedlot disease in the United States. The pathogens that cause BRD are viral, bacterial, or both. *Mannheimia haemolytica* serotype 1 is the most common bacterial pathogen associated with BRD (Pandher, *et al*, 1998). Other bacterial species contributing to the disease can include *Mycoplasma* species, and *Histophilus somni.* Viral agents include infectious bovine rhinotraceitis (IBR), parainfluenza-3 (PI3), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), and bovine enteric coronavirus (Duff & Galyean, 2007).

However, the BRD complex is a multifaceted problem (Duff & Galyean, 2007). Contributing factors such as weaning, marketing, transportation, genetics, and history can have negative effects on immune function (Duff & Galyean, 2007). This stress can be compounded by a decrease in feed intake for up to 3 weeks after cattle arrive at the feedlot (Hutcheson, 1980). At this time, cattle are exposed to many new different infectious agents due to comingling with other animals from different origins (Duff & Galyean, 2007). The stressed and naïve animals are

exposed to new pathogens, leading to an increase in the likelihood of cattle contracting the agents that cause BRD.

While most animals entering a feedlot are vaccinated against the majority of these pathogens, clinical disease can spread very easily in an environment which houses thousands of animals from hundreds of sources at one time. Feedlot staff are trained to recognized these cases of clinical disease throughout the feedlot. Clinical signs of BRD include nasal or ocular discharge, depression, lethargy, emaciated body condition, or labored breathing (Duff & Galyean, 2007). Symptomatic cattle with a rectal temperature ≥39.7°C are usually considered morbid and given therapeutic antibiotic treatment (Duff & Galyan, 2007). Most animals are removed for examination and treatment for BRD on or before d 27 of the receiving period (Buhman *et al*, 2000).

Effects of Implantation on Disease Status

Many cattle are in a state of stress at feedlot arrival and have not yet become accustomed to the environment, feeding routine, etc. These high-risk cattle may have reduced intakes for up to three weeks after arrival at the feedlot (Hutcheson & Cole, 1986). Subsequent immunosuppression leads to an increased risk of bovine respiratory disease (BRD) (Blecha *et al*, 1984).

It has been hypothesized that implanting cattle with steroid implants upon arrival at the feedlot could be detrimental to their health, and therefore to their overall performance because of increased metabolic activity at a time in which they are not consuming feed. When cattle are stressed, such as during or immediately after transport, or during/after routine processing procedures (vaccinating, implanting, castrating, dehorning, etc.), intake is usually depressed (Hutcheson & Cole, 1986). This stress and decreased intake can result in disease, particularly

BRD, which could be exacerbated in implanted cattle compared to non-implanted cattle. (Duff & Gaylean, 2007).

It has been observed that weight loss and decreases in body condition are typical in cattle affected by BRD (Cole *et al*, 1986). Questions to ask include: could the increase in nutrient requirements needed due to anabolic steroid implants have detrimental effects in such a situation? Also, can these anabolic steroid implants in fact create a catabolic state in cattle when the nutrient requirements are not met?

Therefore, the objectives of this thesis are to:

1. Examine the impact that anabolic implants may have on animals in a restricted nutritional state.
2. Evaluate the concentrations of certain signaling proteins and their involvement on myosin heavy chain expression in primary skeletal muscle cell culture
3. Determine if the two previous objectives may be linked in some way on a cellular level, and if that link can be extrapolated to use in decision-making when considering implant strategies in a large commercial feedyard.

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Tables & Figures

Table 1.1: Commercial implants available today.

Category	Trade name	Ingredients	Dosage (mg)	Suitable cattle type
Mild estrogens				
	Compudose[a]	Estradiol	25.7	Steers and heifers
	Encore[b]	Estradiol	43.8	Steers and heifers
	Ralgro[c]	Zeranol	36	All cattle
	Implus-C[d]	Progesterone	100	Steers and heifers
	Component E-C[a]	Progesterone	100	Steers and heifers
	Synovex-C[e]	Estradiol benzoate	10	Steers and heifers
Strong estrogens				
	Ralgro Magnum[c]	Zeranol	72	Steers
	Implus-S[d]	Progesterone	100	Steers
	Component E-S[a]	Progesterone	20	Steers
	Syonvex-S[e]	Estradiol benzoate	20	Steers
Androgens				
	Component T-S[a]	Trenbolone acetate	140	Steers
	Finaplix-H[d]	Trenbolone acetate	200	Heifers
	Component T-H[a]	Trenbolone acetate	200	Heifers
Mild combination				
	Implus-H[d]	Testosterone proprionate Estradiol benzoate	200 20	Heifers
	Synovex-H[e]	Testosterone proprionate Estradiol benzoate	200 20	Heifers
	Component E-H[a]	Testosterone proprionate Estradiol benzoate	200 20	Heifers
	Revalor-S[d]	Trenbolone acetate Estradiol	120 24	Steers
	Component TE-S[a]	Trenbolone acetate Estradiol	120 24	Steers
	Revalor-H[d]	Trenbolone acetate Estradiol	140 14	Heifers
	Revalor-G[d]	Trenbolone acetate Estradiol	40 8	Steers and heifers
	Revalor-IH[d]	Trenbolone acetate Estradiol	80 8	Heifers
	Revalor-IS[d]	Trenbolone acetate Estradiol	80 16	Steers
	Component TE-G	Trenbolone acetate Estradiol	40 8	Steers and heifers
Strong combination				
	Synovex Plus[e]	Trenbolone acetate Estradiol benzoate	200 28	Feedlot steers and heifers
	Revalor-200[d]	Trenbolone acetate Estradiol benzoate	200 20	Steers
	Revalor-XS[d]	Trenbolone acetate Estradiol	200 40	Feedlot steers and heifers

*Table modified from that listed in Montgomery *et al*, 2001

[a] Elanco Animal Health (Greenfield, IN)

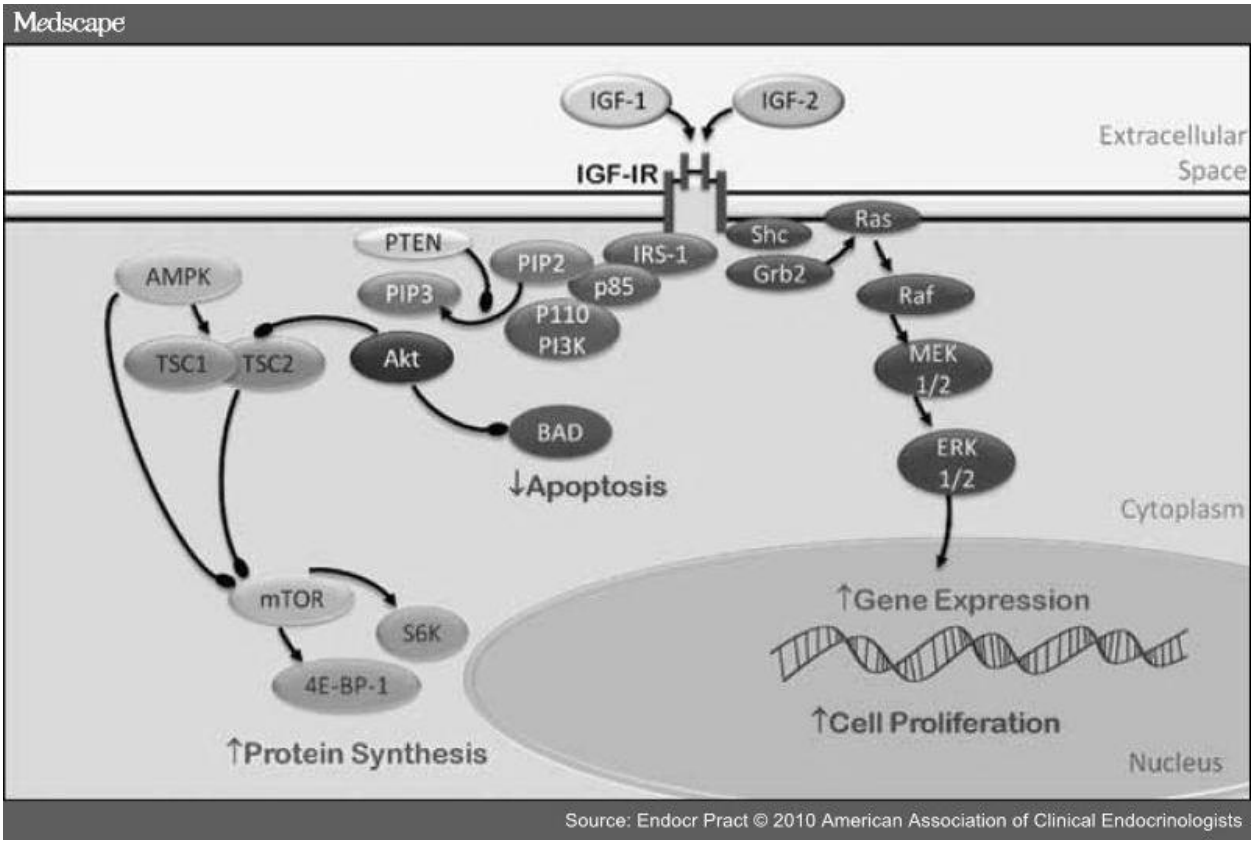
[b] Vet live (Winterset, IA)

cMerck Animal Health (Summit, NJ

[d] Intervet-Schering-Plough (Madison, NJ)

[e] Pfizer Animal Health (Madison, NJ)

Figure 1.1: ERK Cascade



Chapter 2 - The Effects of Nutrient Intake on Steers Administered Anabolic Implants

Introduction

Anabolic implants have been shown to increase average daily gains 15 to 20%, and improve feed efficiency by 6 to 14% in feedlot cattle (Duckett & Andrae, 2001). However, when animals first arrive in a feedlot, many are in a state of stress and have not yet become accustomed to the environment, feeding routine, social structure, and other factors, especially if they have traveled long distances, or have recently been weaned. These cattle are determined to be "high risk" cattle; i.e. they are considered at high risk to develop bovine respiratory disease (BRD). Many of these high-risk cattle have reduced intakes for up to three weeks after arrival at the feedlot (Hutcheson & Cole, 1986), which further predisposes them to develop BRD.

In humans and other non-ruminants, stress and/or infection can lead to a hypermetabolic state, characterized by weight loss, increased resting metabolic rate, and increased muscle protein catabolism (Cole *et al*, 1986). In cattle infected with BRD, decreased feed intake, weight loss, and decreased body condition are often observed (Cole *et al*, 1986). Although ruminants may have a greater capacity to store and utilize nutrients because of their larger and more diverse digestive tracts, this weight loss and decrease in body condition may be comparable to the hypermetabolic state that develops in non-ruminants (Cole *et al*, 1986). In such high-risk cases, it has been suggested that implanting cattle upon arrival at the feedlot could be detrimental to their performance, further increasing stress and complicating existing risk factors. The objective of this study was to examine the effects of anabolic implants on measures of nutrient balance, metabolic status, and growth factors in animals consuming nutrients either adequate or inadequate to support growth.

Materials & Methods

Animals

Sixteen crossbred (predominantly continental breed) steers were purchased locally and transported to Kansas State University, and housed in accordance with university Institutional Animal Care and Use Committee guidelines. They were weighed, tagged, and vaccinated with a 5-way respiratory viral vaccine and a 7-way clostridial vaccine. The steers were trained to individual Calan® gates over a period of 2 weeks.

After training was complete, the steers were randomly assigned to one of four treatments in a 2 × 2 factorial arrangement: (1) administration of an anabolic growth implant (Revalor-XS: 200 mg trenbolone acetate and 40 mg estradiol 17-β; Merck, Whitehouse Station, NJ), and provided 2.0 x maintenance of a moderate energy starting cattle diet; (2) administration of the same implant, but fed the with aforementioned diet at 1.0 × maintenance; (3) no implant administered, and provided 2.0 x maintenance of the same starting diet; (4) no implant and fed at 1.0 × maintenance. Cattle were fed a total mixed pelleted ration (Table 1).

Animals were weighed on d 0, 14, and 28 of the trial, after being fasted overnight. At each of these time points, maintenance requirements were calculated for each steer (NRC, 1984), and rations were fed based these calculations and the treatment assignment of each steer.

Biochemical Assays

Blood samples (serum and plasma) were taken from each animal at d 0, 14, and 28 of the trial and used in determining levels of IGF-1 and plasma urea nitrogen (PUN). The serum collected was also applied to satellite cells which had been previously isolated from the semimembranosus muscle of two non-study steers and frozen in liquid nitrogen. Satellite cells were incubated with serum of individual animals from d 14 and 28 (20% of total media) for 72 h.

Protein abundance of myosin heavy chain (MYH; d 0, 14, and 28), phosphorylated extracellular signal related kinase (pERK; d 0 and 28), and phosphorylated mammalian target of rapamycin (pmTOR; d 0 and 28) was analyzed in differentiated satellite cells to determine effects of implant, intake, and their interaction (applied via the serum). MYH is used as a marker of myotube formation, and pERK and pmTOR are growth factor protein indicators of cell proliferation.

Statistical Analysis

Animal was the experimental unit for all measures. Data were analyzed using the GLM procedure of SAS® (v. 9.1.3; SAS Institute, Cary, NC); IGF-1 and PUN were analyzed using a repeated measures. The independent variables included the main and interactive effects of implant status and intake. Effects were considered significant based on a protected F-test with $\alpha < 0.05$.

Results & Discussion

Body Weight

No significant interactions were noted in the analysis of body weight. There was an increase in the weight of the steers fed the 2 × maintenance diet ($P < 0.01$), but implantation had no significant effect. It is well understood that estrogenic and androgenic implants increase growth by increasing protein accretion and muscling (Foutz et al, 1997). This, in turn results in increased average daily gain (ADG) and increased hot carcass weight (Duckett & Andrae, 2001). However, with the data gathered in this study, the difference in body weight between the study groups cannot be attributed to implantation alone. Only those steers fed at a 2 × maintenance rate gained weight significantly, compared to those fed a 1 × maintenance diet (Figure 2.1). These data demonstrates that diet is the most influential factor contributing to the growth and

over-all well-being of animals in a feedlot setting. The most influential part of red meat production is whether animals reach intake levels sufficient enough to meet production goals; if these intake levels are not reached, this study shows that implantation will not serve as a means to "make up" for the losses incurred. Verde and Trenkle (1987) demonstrated that there are some differences in hormone concentrations (including growth hormone, insulin, and T4) in plasma from cattle that appear to be due to genetic size, as well as age. The unknown background and breeding of the cattle used in this study may have been a contributing factor to the differences in weight gain between the four groups over the 28-day period, as well as in normal, non-study cattle found in feedlots across the U.S.

Average daily gain also did not follow the patterns seen in previous literature—while not statistically significant, implanted animals fed at 2 × maintenance actually had lower ADG than non-implanted animals fed the same amount. The opposite was observed in those groups fed at a 1 × maintenance diet, but this was again, not statistically significant. The reasons stated above may also have been the cause of such unconventional findings in average daily gain data.

Plasma Urea Nitrogen

There was a tendency for an interaction between diet and implant ($P = 0.09$), showing increased PUN values in steers fed a 2 × maintenance ration, without an implant. Diet alone had a significant impact on PUN levels ($P < 0.01$). According to Thomson *et al.* (1996), there are three ways in which the amount of nitrogen in the plasma of an animal can be changed: changes in protein synthesis, changes in protein degradation, or both. Anabolic growth implants affect the element of protein synthesis in the case of beef cattle (Trenkle *et al*, 1987). It does not increase or decrease the amount of nitrogen retained in the body, but rather increases the utilization of that nitrogen for the use of the aforementioned synthesis of protein (Thomson *et al*,

1996). Because more of the body's nitrogen store is being used in those implanted animals, it has been shown that urea nitrogen levels in the plasma of implanted steers versus non-implanted steers is lower (Thomson *et al*, 1996). However, in the current study, PUN levels of implanted steers versus non-implanted steers were no different (Figure 2.2). When measurements of PUN made by Thomson and others (1996) are compared to data from the current study, nitrogen levels were lower in implanted cattle in the study performed by Thomson, while implanted cattle in the current study showed higher PUN levels. Differences between these data sets includes units of measurement of PUN, and time intervals between measurements, however, when comparing the data, PUN levels should follow a similar pattern, regardless of unit of measurement. It may be speculated that nutrient availability had a significant biological effect on nitrogen retention, but the effect was not seen statistically.

Insulin-like Growth Factor-1 (IGF-1)

In the current study, no significant interactions were noted between diet and implant in the analysis of IGF-1 levels. However, diet had an effect on IGF-1 levels ($P < 0.01$), with increased IGF-1 levels in steers fed 2 × maintenance (Figure 2.3). Because IGF-1 is a mediator of growth promotion, it is intuitive that IGF-1 levels are increased in cattle fed a 2 × maintenance ration. Elasser *et al.* (1989) showed that diet composition and intake have an effect on the circulating plasma IGF-1 levels in growing steers. However, in the current study, the IGF-1 concentrations in implanted cattle show no difference from those in non-implanted cattle.

IGF-1 is a peptide hormone synthesized and secreted mainly from the liver, and mediates the growth-promoting actions of growth hormone (Hong & Forsberg, 1994). The anabolic effects of IGF-1 on skeletal muscle cells and satellite cells include stimulation of amino acid

uptake, protein synthesis, glucose uptake, DNA and RNA synthesis, cell proliferation, and cell differentiation (Hong & Forsberg, 1994, Machida & Booth, 2004).

Johnson and others (1996) concluded that circulating levels of IGF-1 were increased in steers implanted with a combined TBA/E2 implant as compared to control steers given no implant. It has also been shown that a combined TBA and estradiol implant can increase the time in which satellite cells become activated in culture (Johnson *et al*, 1998). An increase in IGF-1 production and satellite cell activation of implanted cattle contributes to the positive effects of implantation on enhancing protein accretion in growing true muscle cells (Johnson *et al*, 1998). However, again, in the current study, the IGF-1 concentrations in implanted cattle show no difference from those in non-implanted cattle.

Myosin Heavy Chain (MYH)

When serum from study animals was applied to cultured cells from the semimembranosus muscle from a non-study steer, intake had no effect on MYH but implant increased MYH abundance ($P<0.01$) (Figure 2.4). This demonstrates that when muscle cells are provided adequate nutrition, implant status does contribute to the amount of protein deposition that is seen within muscle cells, both *in vitro* and *in vivo*.

The growth of skeletal muscle tissue involves a unique and complex series of events. Postnatal skeletal muscle growth can be attributed to both an increase in diameter and length of the muscle fibers. Increase in muscle cell growth is defined as hypertrophy (Aberle *et al*, 1989). Multiple studies have shown that, postnatally, there is an increase in the amount of DNA content in the muscle fibers of various mammals (Winick & Noble 1966, Moss 1968, Johns & Bergen 1976,). This external source of DNA is the muscle satellite cell (Moss & LeBlond, 1970).

However, satellite cells are not in a continuous state of proliferation. They reside in a state of quiescence, and must be stimulated by hormones to progress through the cell cycle. It has been found that the progression factor insulin-like growth factor 1 (IGF-1) is a stimulant of this proliferation (Johnson *et al*, 1998). The results of this study do not show the effects of implantation on circulating IGF-1 factors and subsequent increases in muscle hypertrophy *in vivo*, but do, however, show that skeletal muscle hypertrophy can be seen when serum from implanted steers is combined with cultured myocytes in a controlled and nutrient-adequate environment (*in vitro*).

Abundance of pERK and mTOR

As previously mentioned, cellular growth and proliferation involves a complex series of events that start with signaling at the molecular level. Some of the molecules and proteins that could be synthesized or activated in response to the administration of anabolic steroids include the mammalian target of rapamycin (mTOR) and extracellular signal-related kinases (ERK). The phosphorylation of these signaling proteins may indicate the "turning on" or "turning off" of cellular pathways that increase muscle protein synthesis, and therefore, muscle growth.

The extracellular signal-related kinase (ERK) plays an important role in cell proliferation and differentiation (Lai *et al*, 2001). The ERK cascade is the first of many signaling pathways to be identified in the MAP kinase family, which regulates a number of different physiologic processes within mammalian cells, including cell proliferation and differentiation, hypertrophy, apoptosis, and inflammation (Sakomoto & Goodyear, 2002). This cascade, shown in Figure 2.5, is activated by growth factors, including IGF-1 (Glass, 2005). Because implant status has been shown to have an effect upon IGF-1 levels, it may be speculated that changes in IGF-1 levels caused by implants may result in subsequent changes in phosphorylated ERK levels in skeletal

muscle cells. In the case of the data collected from this set of cattle, implant increased the expression of pERK ($P<0.01$), but intake had no effect, and there was no interaction between the two treatments (Figure 2.6).

The protein kinase mTOR has been shown to play an essential part in the regulation of many cellular activities, including transcription, translation, cell size, and protein stability (Corradetti & Guan, 2006). To help regulate these processes, mTOR integrates information about nutrient status, energy status, and many growth factors (Sorbassov *et al*, 2005). According to Sorbassov *et al.* (2005), mTOR is a central component in the regulation of cellular processes that determine cell growth and proliferation, and even overall animal size. It is because of this integration and the effects on so many different cellular activities that mTOR could be helpful in determining the effects of implantation and nutrient availability in beef cattle.

In the current study, serum from steers with 2 × maitenance intake and implant administration showed increased abundance of pmTOR ($P=0.02$) in cultured myocytes (Figure 2.7). There was an interaction between diet and implant on the expression of mTOR. Intuitively, steers receiving a 2 × maintenance diet showed higher levels of mTOR expression than those receiving maintenance alone, supporting the idea that mTOR is more abundant when a nutrient-adequate environment is present, and cellular growth and proliferation is favored. However, implant alone had no effect on pmTOR at restricted intake ($P=0.37$, interaction $P \leq 0.02$). Interestingly, steers fed at 1 × maintenance had similar mTOR levels, regardless of implant status, which is not to be expected after reviewing the current literature. Phopsphorylated (activated) mTOR is a molecule that has been shown to increase in abundance when nutrition is adequate, and actually decrease when the nutrient environment is not favorable for growth (Coradetti & Gaun, 2006). In the current study, mTOR values were similar in groups

of non-implanted steers fed both 1 and 2 × maintenance diets, which is inconsistent with the current theories involving the abundance of mTOR in relation to nutrition status.

Conclusion

Results of this study show that nutrient status, above all, is the most influential factor in the growth of skeletal muscle in cattle raised for red meat production. With the improvements in efficiency and gain that anabolic growth implants have been shown to provide, and the absence of negative effects of implants as shown in this study, it may be speculated that implantation does not have a negative, or catabolic, effect on skeletal muscle cells when it comes to proliferation and differentiation, even in animals in a restricted nutritional state. The molecular and cellular measurements made in this study have shown that implantation can improve skeletal muscle hypertrophy, but only in the presence of adequate nutrition.

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Figures and Tables

Table 2.1: Composition of grower's ration

Wheat middlings	14.68
Cracked corn	10.73
Corn gluten feed	18.22
Extender pellets	41.82
Cottonseed hulls	7.68
Dried distiller's grain	3.01
Molasses	1.67
Limestone	1.85

Diet also included salt, zinc sulfate, vitamin A, and Rumensin 80

Total Diet Composition	
CP	15.3
Ca	0.56
P	1.43
NEm (Mcal/kg)	1.44
NEg (Mcal/kg)	0.85

Table 2.2: ADG for each trial goup

	Day 14	**Day 28**
1 × Maintenance, Implant	0.0	0.3
1 × Maintenance, No Implant	-0.8	-0.1
2 × Maintenance, Implant	4.5	3.6
2 × Maintenance, No Implant	5.5	3.6
SEM	1.47	0.94

Figure 2.1: Body weight by diet and implant at day 14. Points not bearing a common letter differ. Diet had a significant effect on body weight (P < 0.0), however implant did not (P = 0.45). No interaction was observed (P = 0.23).

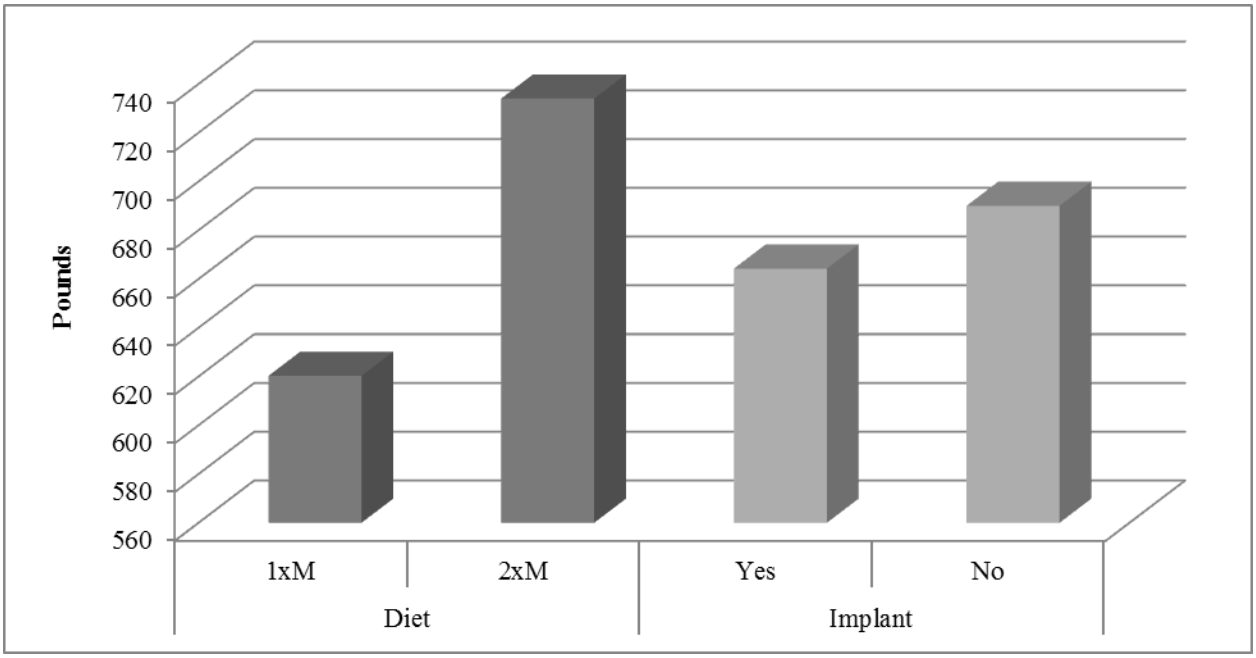

Figure 2.2: PUN concentration of steers implanted with a long-term TBA:E2 implant. There was a tendency for diet to have an effect on implant effects (P = 0.09). Points not bearing a common letter differ.

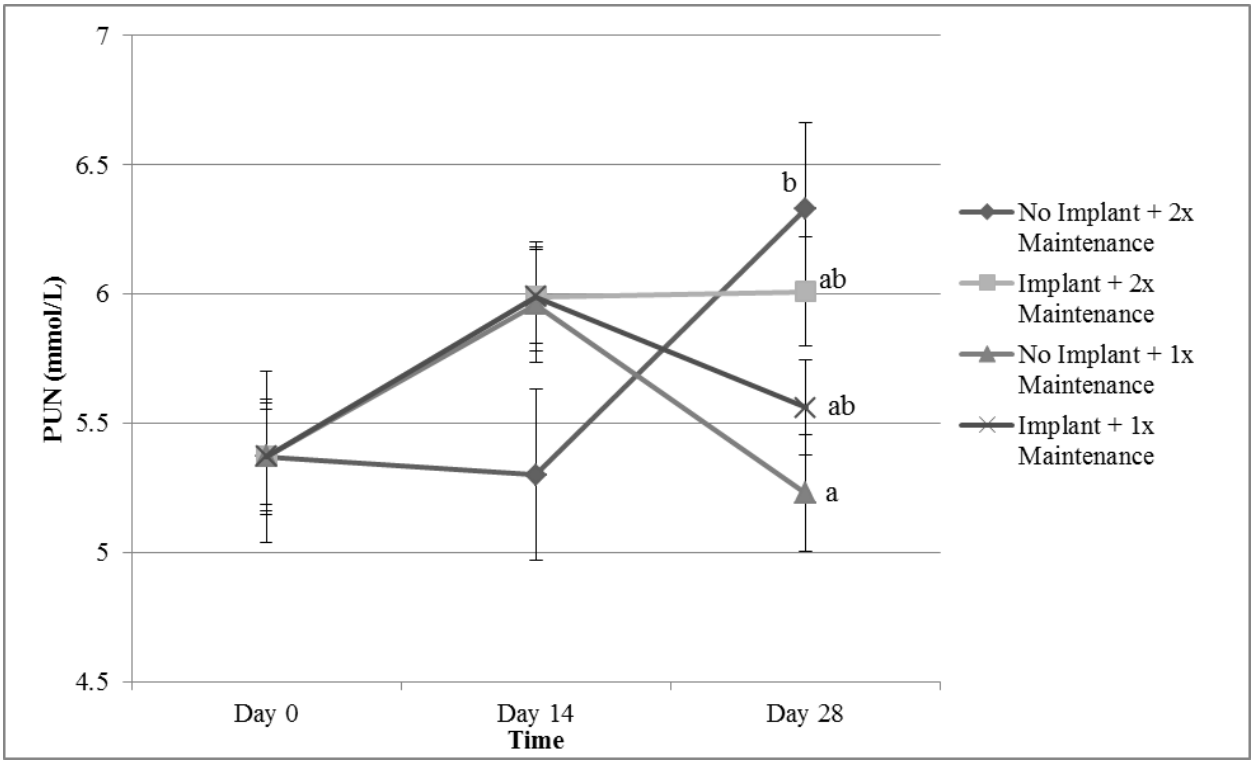

Figure 2.3: IGF-1 concentration in steers by diet and implant. Points not bearing a common letter differ. There was no diet × implant interaction (P = 0.33), implant did not affect IGF-1 concentrations (P = 0.41), however nutrient status seemed to increase IGF-1 concentrations in animals fed 2 × maintenance diet (P < 0.01).

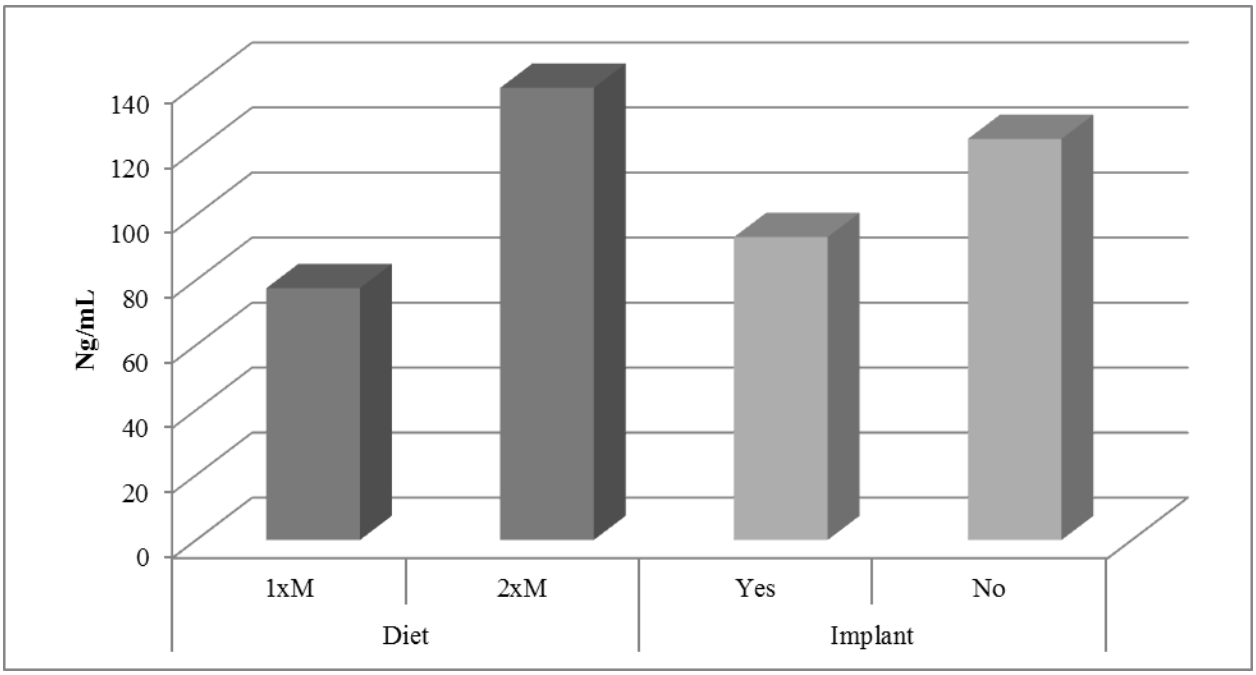

Figure 2.4: Myosin heavy chain abundance in cultured myocytes from a non-study steer. Myocytes were treated with serum from study steers on days 28, and myosin heavy chain abundance was measured. Serum from implanted cattle caused an increase in the MYH abundance compared to cells treated with serum from non-implanted cattle (P < 0.01), however, diet had no effect (P = 0.85). Points not bearing a common letter differ.

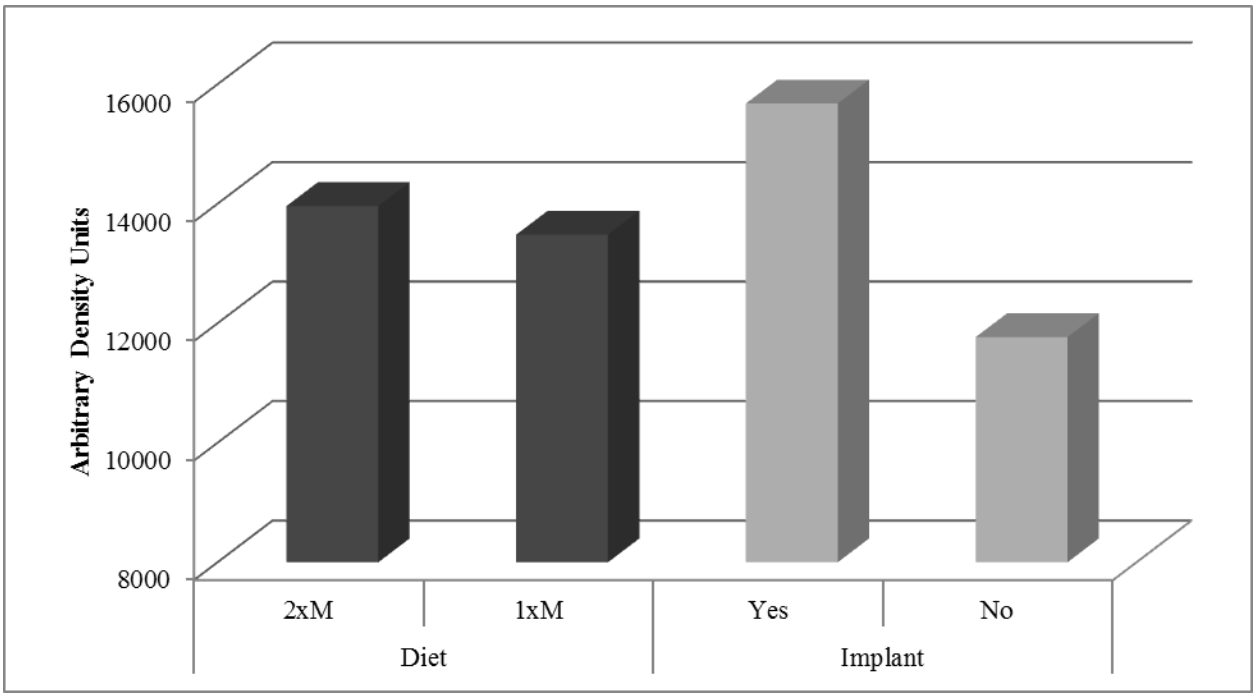

Figure 2.5: ERK cascade

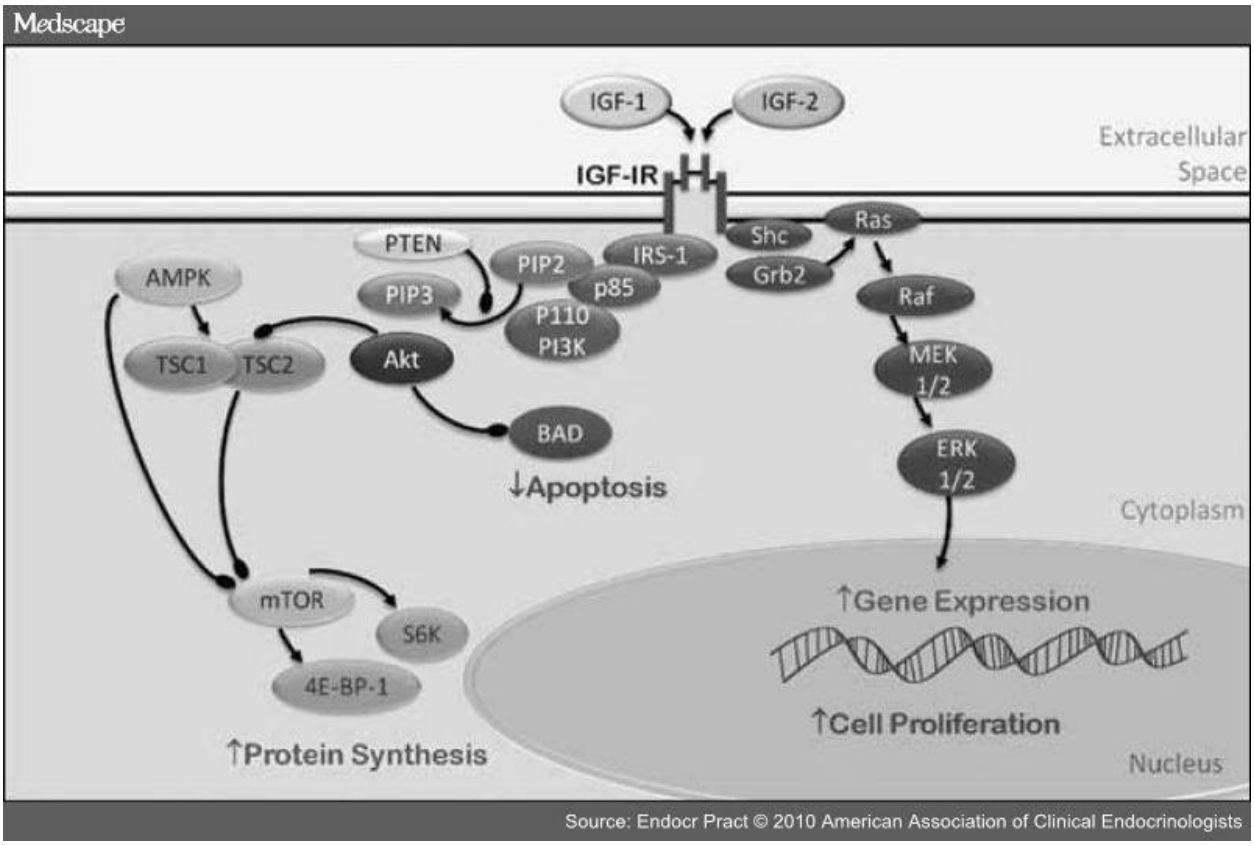

Figure 2.6: Abundance of phosphorylated ERK (in arbitrary density units) in cultured myocytes receiving serum from study steers. Implant increased pERK levels in cattle, regardless of nutrient status (P < 0.01). SEM = 556.2.

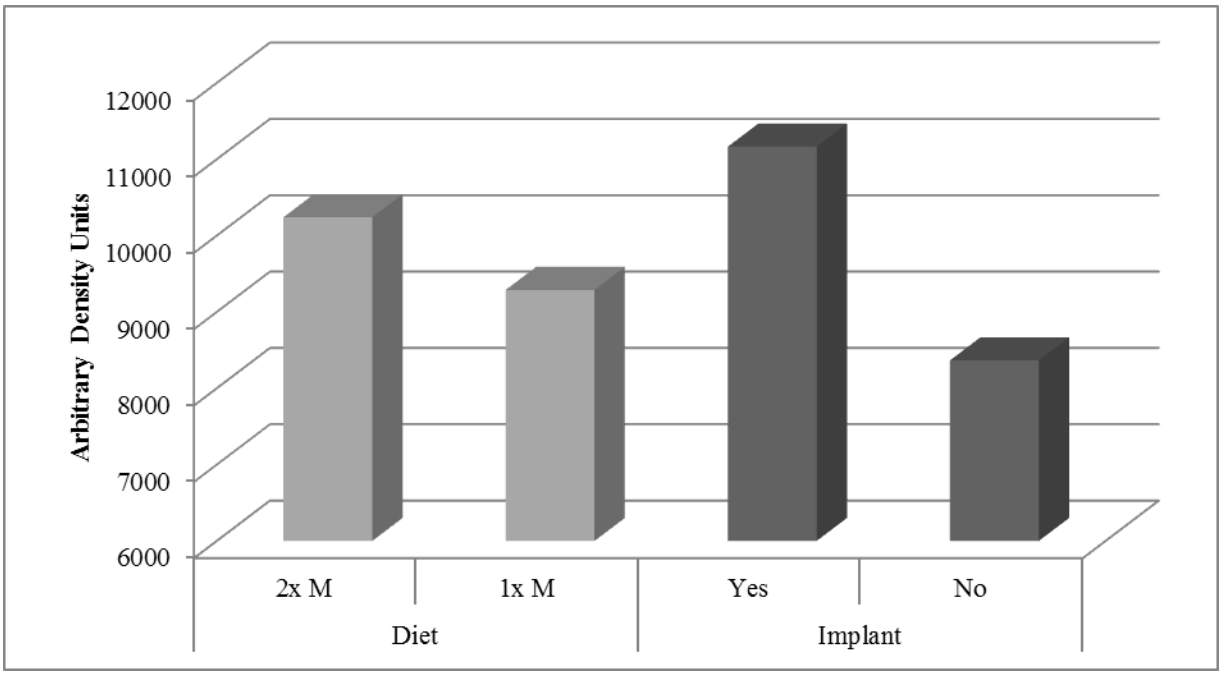

Figure 2.7: Abundance of phosphorylated mTOR (in arbitrary density units) in cultured myocytes receiving serum from study steers. Diet (2 × maintenance = lighter grey) showed a significant effect on the mTOR levels in implanted steers ($P \leq 0.02$). SEM = 609.2.